Proceedings of the Indian Academy of Sciences
(Plant Sciences)

Volume 94, 1985

CONTENTS

The structure of the pod in two species of *Prosopis*
S Rangayya, I L Kothari and G L Shah 1

Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memoriam optimi Professoris Swamy *E Govindarajalu* 11

Factors controlling callus initiation, growth and plant regeneration in bread-wheat (*Triticum aestivum* L.) *Susan Eapen and P S Rao* 33

Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species *N V R Reddy and K R Rao* 41

Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae *T A Sarma and Raj Garg* 45

Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae *V V Sivarajan and M C Gopinathan* 51

Nitrogen fixation by *Nostoc* strains
N Jayasankari and S Shanmugasundaram 59

Pharmacognostic studies on 'Dravanti' part-I *Jatropha curcas* Linn
R C Gupta 65

C. V. Subramanian 60th Birthday Felicitation Volume

Dedication 83

Effect of soil texture on microbial abbreviation of saprophytic survival by the take-all fungus of wheat *S D Garrett* 85

Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia *K Sivasithamparam and I Rowland* 91

Specificity and penetration of tissues by micorrhizal fungi *J L Harley* 99

Factors affecting the occurrence of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with roots of trees *F T Last and L V Fleming* 111

Fungal endophytes of forage grasses *Garry T Cole and James F White* 129

Ambrosia beetles and their associated fungi: research trends and techniques
L R Batra 137

Ecology of keratinophilic fungi
A P Garg, Sudha Gandotra, K G Mukerji and G J F Pugh 149

Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) *Gilbert Turian* 1

Enzymes from thermophilic fungi: Proteases and lipases *Bhavdish N Johri, Sanjay Jain and Satish Chouhan* 1

Physiological studies of the Gymnoascaceae with an overview on their ecology *Gouri Rani Ghosh* 1

Conidia as modified hyphae *E Descals* 2

Notes on some deuteromycete genera with cheiroid or digitate brown conidia *B C Sutton* 2

On the anamorph genus *Cirrenalia* *R D Goos* 2

Porosubramaniana, a new genus of lignicolous hyphomycetes *Věra Holubová-Jechová* 2

Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete *Nathalie Buffin and G L Hennebert* 2

Two new hyphomycetes from *Ethiopia* *D J Bhat* 2

Pigment localization in Dematiaceous hyphomycetes and the segregation of *Pseudogliomastix* gen. nov. from *Acremonium* *W Gams and T Boekhout* 2

A re-appraisal of the relationships between *Arthrinium* and other hyphomycetes *David William Minter* 2

New and rare entomogenous fungi from Amazonia *Robert A Samson and Harry C Evans* 3

Problems and prospects in the systematics of the Ascomycotina *D L Hawksworth* 3

On *Achaetomium* and a new genus *Subramaniula* (Ascomycota) *J A von Arx* 3

Meliolina subramanianii sp. nov *S J Hughes and K A Pirozynski* 3

Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* *Jan Kohlmeyer* 3

Pseudascozonus, a new genus of Pezizales *J van Brummelen* 3

The species concept in Hymenochaetaceae (Fungi, Hymenomycetes) *E Parmasto* 3

The relevance of chemical characters for the taxonomy of Agaricales *Meinhard Moser* 3

Monitoring of wheat rusts in the Indian sub-continent *L M Joshi, K D Srivastava and D V Singh* 3

Identity of dactuliophora leaf spot of pearl millet *P D Tyagi* 4

Management of *Rhizoctonia solani* diseases with chemicals *Rajendra K Grover and Hans R Kataria* 4

Cylindrocarpon species associated with mycotic keratitis
C Booth, Y M Clayton and M Usherwood 433

Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice
M Miyaji and K Nishimura 437

Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals
Edouard Drouhet, Claude de Bievre and Seema Wahab 453

India's changing agriculture: Its management, genetic vulnerability and diversification *H K Jain* 465

Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue
Wilhelm Nultsch and Maria Häder 475

Additional DNA sequences—Topography, property, role and evolutionary significance *Arun Kumar Sharma* 487

Strand plant communities of the Indian sub-continent
T Ananda Rao and V M Meher-Homji 505

Floral differentiation and its modification *I Usha Rao and H Y Mohan Ram* 525

Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation
Shubhada Patankar, C P Joshi, S A Ranade, Mrinal Bhave and P K Ranjekar 539

Development of male and female gametophytes and integument initiation in *Prenanthes brunoniana* Wall. (Compositae)
Sarvesh Kumar Sood and Tejinder Singh Thakur 553

Significance of seed coat pattern in *Vigna radiata* var. *sublobata*
S Ignacimuthu and C R Babu 561

Survey of some Indian soils for laccase producing fungi and their lignin degrading ability *D S Arora and D K Sandhu* 567

Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils *R Kasturi Bai and P N Raju* 575

Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) *G R Hegde* 583

Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.)—I. Stem-bark and root *R C Gupta* 589

Cytology of woody species *V K Singhal, B S Gill and S S Bir* 607

Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) *S S Bir and M I S Saggoo* 619

Seasonal growth, reproduction and spore shedding in *Pterocladia heteroplatos*
N Kaliaperumal and M Umamaheswara Rao 627

Seedling anatomy of *Brassica campestris* L. *S Babber and A Pillai* 633

The veinlet syndrome in the tribe Andromedeae (Ericaceae)
T Ananda Rao and Swapna Chakraborti 639

Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight *T Ananda Rao, Swapna Chakraborti and Silpi Das* 655

Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations *Karan Singh and Y S Murty* 663

Foliar application of potassium on the growth and yield components of pigeonpea (*Cajanus cajan* (L.) Mill sp.)
N N V S Ravindranath, N V Satyanarayana, P Prasad and K V Madhava Rao 671

The pericarpic structure of *Withania somnifera* (L.) Dunal
Y S Dave, N D Patel and A R S Menon 677

Effect of different growth substances on the foliar stomata of *Tagetes erecta* L.
Vidhu, R K Sharma and Y S Murty 683

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 1–9.
© Printed in India.

The structure of the pod in two species of *Prosopis*

S RANGAYYA, I L KOTHARI and G L SHAH
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India

MS received 23 March 1984

Abstract. The structure of the stipe and basal, middle and apical regions of the pod is described. The older stipe shows secondary growth as in a dicotyledonous stem. The stipe at its junction with the valves shows three vascular bundles, one large and two small, arranged in a triangular manner, with 3–5 lateral bundles in *Prosopis juliflora*. The mature pericarp of *P. cineraria* has one dorsal and two ventral vascular sutural bundles, but in *P. juliflora* there is a pair of vascular bundles in each suture. The valvular structure can be distinguished into one layered epicarp, multilayered mesocarp and few to many layered endocarp. The outer mesocarpic layers and all endocarpic layers are sclerenchymatous in *P. juliflora* but only the endocarpic layers are collenchymatous in *P. cineraria*. Within the circumscription of structural types of pod described by Fahn and Zohary (1955), the *Trifolium* type (form d) is found in *P. cineraria* and the *Cyanophyllum* type in *P. juliflora*. Taxonomic importance of anatomical characters is brought to light to separate the two taxa.

Keywords. *Prosopis*; pod structure; taxonomic significance.

1. Introduction

As far as the Mimosaceae is concerned our knowledge about the mature pod structure is limited to only about twelve species out of nearly a hundred leguminous taxa investigated by Fahn and Zohary (1955). Therefore, a detailed study of the pod structure at basal, middle and apical regions is undertaken in some members of the Mimosaceae during different stages of development to supplement the existing data. Our observations are recorded on *Calliandra tweedii* (Shah *et al* 1983), *Leucaena leucophloea* (Shah and Rangayya 1983), *Desmanthus virgatus* (Shah and Rangayya 1984) and *Neptunia* (Rangayya and Shah 1984). In this paper, our observations on the pods of *Prosopis cineraria* Druce and *P. juliflora* (L.) Sw. are presented.

2. Material and methods

P. cineraria (L.) Druce is indigenous in scrub forests, much used as a fodder, the wood as fuel and tender pods as vegetables or medicinally as an applicant in acute headache. *P. juliflora* (L.) Sw. is an introduction from Mexico or Central America primarily to check desert shifting and for soil reclamation. This too is a good fodder and fuel plant. Both the species locally grow wild. The stipe, middle region and apex of mature pods, in various stages of development, were fixed in FAA for 48 hr and, thereafter, stored in 70% ethyl alcohol. The fixed material was dehydrated, embedded in paraffin and serial microtome sections (8–10 μm thick) stained with safranin and fast green were made permanent following the usual procedures (Jensen 1962).

3. Observations

3.1 *Morphology of pod*

The mature pod in *P. cineraria* is 10–15-seeded, deep brown, straight, cylindric, turgid, weakly apiculate, indehiscent and very shortly stipitate (0·3–0·5 cm long). On the other hand in *P. juliflora* it is 20–25-seeded, somewhat falcate, thick, fleshy, cylindrical with tough, leathery but slightly compressed valves, 0·3–0·5 cm long, stout, triangular apex and 1–1·5 cm long terete stipe.

3.2 *Internal structure*

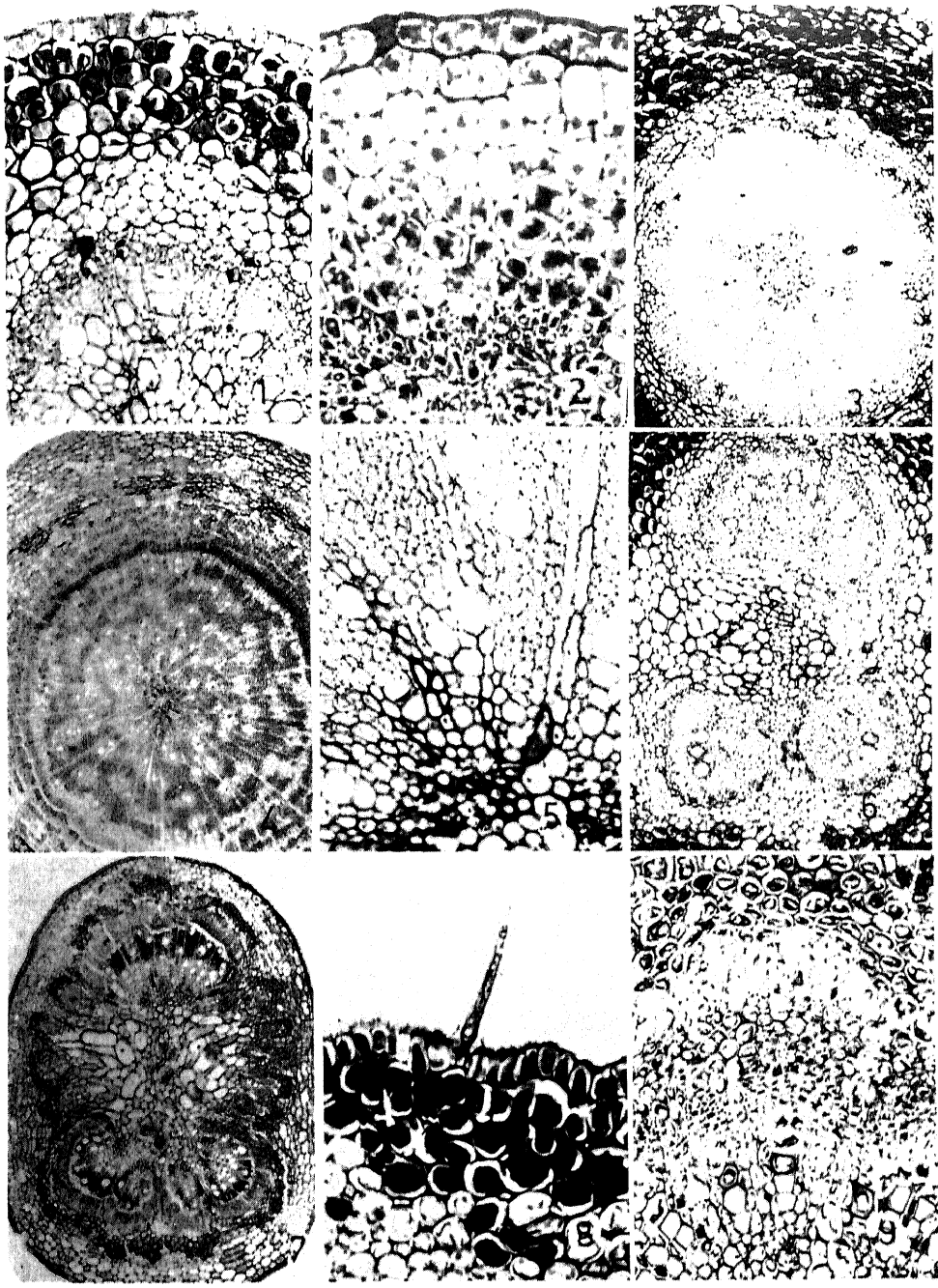
3.2a *Stipe*: In young stipe the epidermis is one layered, cells squarish rectangular, columnar or oblong, vacuolated, nucleated, thin walled except for the moderately thick and rough or smooth cuticularized rounded or flat outer walls. Cortex is compactly parenchymatous, cells thin-walled with dense cytoplasm and nucleus, spherical, oval or oblong in *P. cineraria* and mostly polygonal in *P. juliflora* (figures 1, 2). 5–6 hypodermal layers richly tanniniferous, and larger than the cells of inner layers delimite the stele in *P. cineraria* (figure 1). Vascular bundles are conjoint, collateral, endarch and open in a somewhat uneven ring enclosing a broad-parenchyma. Tracheary elements are spherical to angular in radial rows separated by thin walled xylem parenchyma (figures 1, 2).

As the pod matures the secondary vascular tissues are formed in the stipe with a reduction in size of the pith. Tracheary elements may be diffuse or radially separated by moderately thick walled xylem parenchyma (figures 3, 4) or xylem fibres (figures 4, 5). Medullary rays are uni- or biseriate of thin to moderately thick-walled parenchymatous cells (figures 3–5). Phloem consists of sieve tubes, companion cells and phloem parenchyma, with tanniniferous cells in *P. cineraria* (figure 3) and scattered patches of phloem fibres in *P. juliflora* (figure 4). Cambium is 2–3 layered in the former and many layered in the latter.

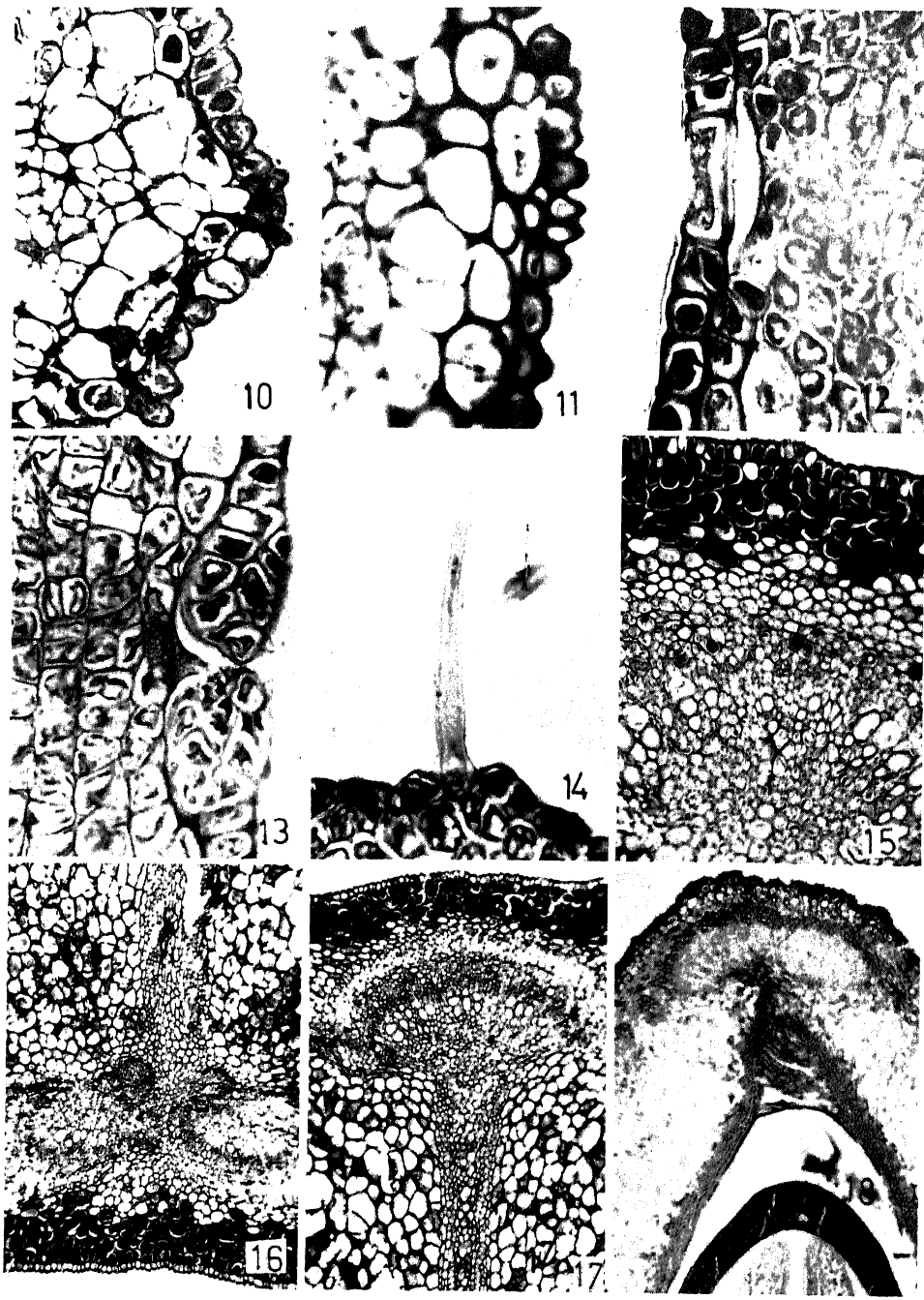
There are no concomitant changes in the extra-stelar region in both the species. A transection of the mature stipe in *P. cineraria* at the junction of pericarpial valve shows three vascular bundles, one larger than the other two arranged triangularly each without sclerenchymatous sheath (figure 6). A similar arrangement is also observed in *P. juliflora*, but each of the smaller bundles is a pair of unequal bundles. In addition there are 3–5 lateral bundles between the large and small bundles. All the bundles have an almost semilunar sclerenchymatous sheath on the outer side (figure 7). Tracheary elements are angular, moderately thick-walled and in radial rows. Phloem 3–5 layered, some peripheral phloem cells in *P. cineraria* containing tannin (figure

3.2b *Middle region of the pericarp*: It can be distinguished into a one layered outer epidermis-epicarp, the middle region—the mesocarp and the endocarp lines the seed chamber.

The outer epidermis in the young pericarp consists of squarish, rectangular columnar, thin-walled cells with flat or rounded outer walls which are thick-walled and moderately thickly cuticularized in *P. cineraria* (figure 8) but unthickened and thinly cuticularized in *P. juliflora* (figure 9). As the pericarp matures the outer walls often become papillate or protruberant, more thickened and heavily cuticularized



Figures 1–9. Transections of basal region. **1, 3, 6, 8.** *P. cineraria*; **2, 4, 5, 7, 9.** *P. juliflora*. **1.** Young pod. (× 85). **2.** Outer region of young pod (× 366). **3.** Mature pod. (× 229). **4.** Extreme base of mature pod (× 37). **5.** Vascular region of mature pod. (× 229). **6, 7.** Basal region (**6** × 229; 7 × 37). **8.** Eglandular trichome (× 366). **9.** Dorsal suture of maturing pod. (× 229).



Figures 10–18. Transections of middle region. **10, 15–17.** *P. cineraria;* **11–14, 18.** *P. juliflora.* **10, 11.** Epidermis showing cuticle (× 366). **12.** Stoma in longisectional view (× 366). **13.** Stoma in transectional view. Note periclinal divisions in neighbouring cells (× 366). **14.** Eglandular trichome. (× 366). **15.** Dorsal suture of maturing pericarp (× 229). **16.** Ventral suture of mature pericarp (× 85). **17, 18.** Dorsal suture of mature pericarp (**17** × 85; **18** × 37).

P. cineraria (figures 8, 10), but the cells are thickened all round in *P. juliflora* (figure 11). Even then the cell contents are seen in both the species.

Stomata are observed only in young and some early stages of maturing pericarp. They are situated at the level of other epidermal cells. Their orientation is irregular as can be judged by some stomata being cut transversely and others longitudinally (figures 12, 13). Guard cells are elliptic or oblong and slightly thick walled in *P. cineraria* but they are ovate or triangular and thickened on the inner walls supported by a cushion of 6–10 thick-walled compact cells in *P. juliflora* (figure 13). The inner and outer ledges are seen in both the species (figure 13).

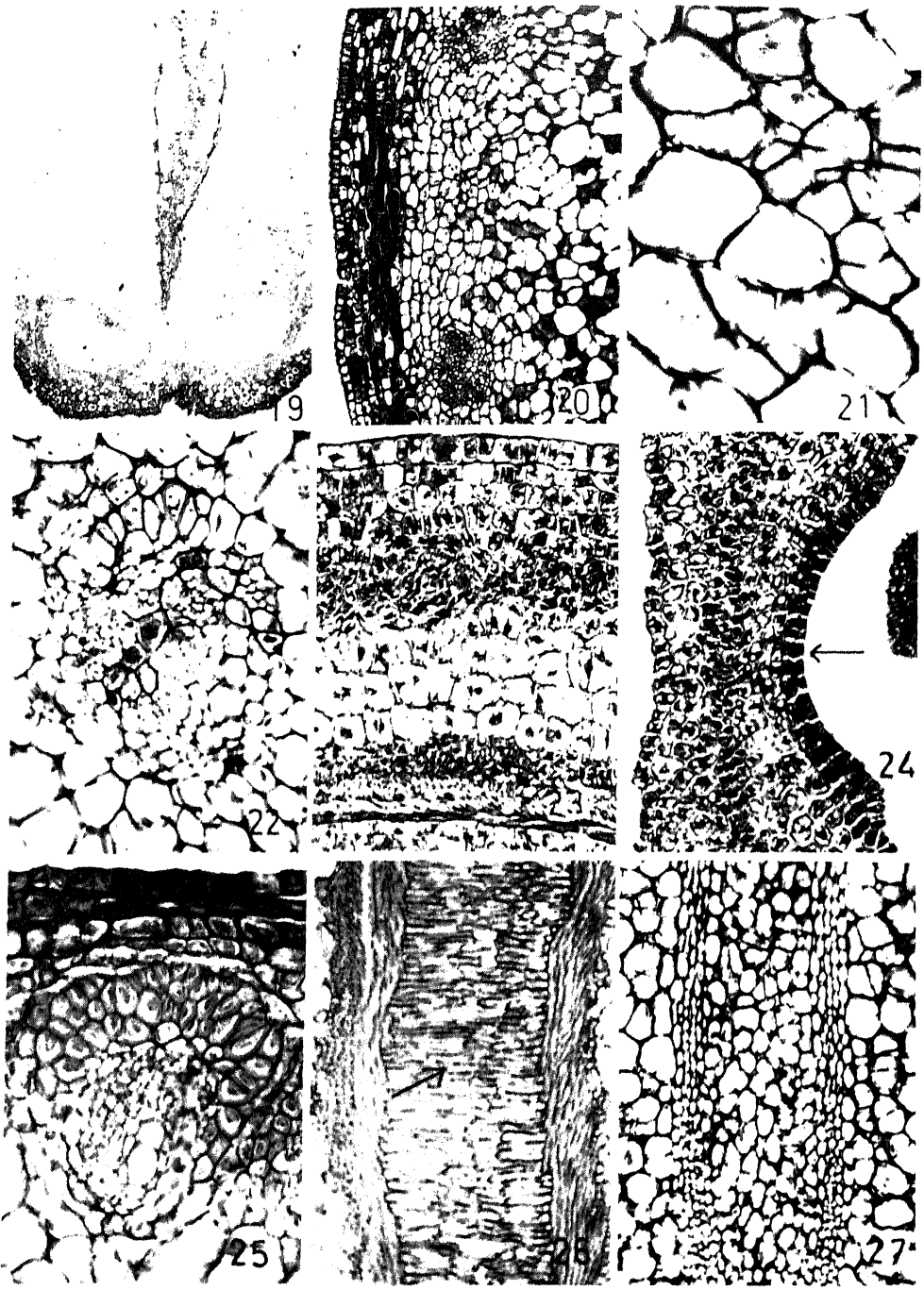
Trichomes are sparingly seen on the young pericarps. They are eglandular, unicellular, conical, thick-walled with smooth cuticle and broad lumen with vacuolated contents (figures 8, 14).

Mesocarp in the sutural region between the outer epidermis and the vascular bundles is 8–15 layered parenchymatous, of thin-walled, spherical or oblong cells, those of 4–5 hypodermal layers are larger than those of the remaining layers, delimiting the sutural vascular bundles. In *P. cineraria* the hypodermal layers are richly tanniniferous (figures 15–17). The number of vascular bundles does not change in young and mature pericarps. In *P. cineraria* there is one bundle in the dorsal suture and two in the ventral one (figures 16, 17) but in *P. juliflora* there are a pair of bundles in each suture (figures 18, 19). Radial tracheary elements are separated by thick-walled lignified xylem parenchyma. Phloem is several layered with a few cells tanniniferous in *P. cineraria*. Cambium is 3–4 layered (figures 15–19). In *P. juliflora*, 3–4 layered broadly semilunar sclerenchymatous sheath is present above the dorsal and ventral sutural bundles (figures 18, 19).

Valvular mesocarp in the mature pericarp of *P. cineraria* consists of a richly tanniniferous outer zone of 6–8 hypodermal layers of tangentially elongated cells separated from the rest of the non-tanniniferous zone. The 4–5 layers below the tanniniferous zone and in the vicinity of the vascular bundles are also tangentially elongated. The cells of the rest of the mesocarpic layers are rectangular, polygonal, oval or oblong, irregularly oriented, with a few small intercellular spaces or without them (figure 20). Some cells become thick-walled, pitted and are scattered or in groups of 2–3 (figure 21). Valvular vascular bundles differentiate in young pericarp. They are 10–12 in the mature pericarp, each with a 2–3 layered sclerenchymatous cap above it (figure 22). Tracheary elements are angular in radial rows, separated by thin-walled xylem parenchyma. Phloem is in 2–3 small groups. Cambium is not distinct.

The valvular mesocarpic structure in *P. juliflora* is different from that in *P. cineraria*. In the young pericarp it is parenchymatous, thin-walled, outermost 2–3 layers consist of squarish or rectangular cells with vacuolated cytoplasm and nucleus. The cells of the next 8–10 layers constituting the middle region are smallest in size, with dense cytoplasm and nucleus. The cells of the innermost region of 4–5 layers are largest, squarish, rectangular or polygonal, sometimes spherical, all thin-walled, highly vacuolated, each with a nucleus. The cells of the outer and inner mesocarpic layers are arranged in almost regular files whereas those of the middle layers are irregularly oriented (figure 23). As the pericarp matures the cells of the outer mesocarpic layers become sclerenchymatous (figure 31) but those of the innermost layers become elongated radially, occupy the major portion of the pericarp and are filled with mucilage (figure 32).

Valvular vascular bundles are 30–40 in the middle mesocarpic layers in the mature



Figures 19–27. Transections of middle region. **20–22, 27.** *P. cineraria*; **19, 23–26.** *P. juliflora*. **19, 20.** Ventral region of mature pericarp. (**19** × 37; **20** × 85). **21.** Inner mesocarpic cells showing pit fields (× 366). **22, 25.** Valvular vascular bundle of mature pericarp (**22** × 366; **25** × 229). **23, 24.** Valvular region of young pericarp. Inner epidermis at arrow in **24** (× 229). **26.** Endocarp of mature pericarp. Pseudoparenchyma at arrow (× 85). **27.** Endocarp of maturing pericarp (× 229).

valve. They are broadly wedge-shaped, conjoint, collateral, endarch with a 2–3 layered hemispheric sclerenchymatous sheath above it. Tracheary elements are angular in radial rows separated by thin-walled xylem parenchyma (figure 25). The vascular zone together with the thick-walled outer mesocarpic layers and epicarp forms a tough and leathery skin of the pericarp.

Endocarp in *P. cineraria* is 4–5 layered. The inner epidermis lines the seed cavity. It is a layer of thin-walled, columnar cells with dense cytoplasm and nucleus. The cells of the outer layers are compact and tangentially elongated (figure 26). In subsequent stages of development they become collenchymatous and the inner epidermal cells of the two valves variously divide to form a loose parenchymatous tissue in the inter-seed region (figure 27). In *P. juliflora* it is 3–4 layered in the young pericarp, the inner epidermis is one layered; cells of all layers are rectangular or squarish, densely cytoplasmic, each with a nucleus (figure 24). In subsequent changes of development numerical and structural changes take place in these layers. In the mature valve all the layers become sclerenchymatous, cells of the outer 3–4 layers are radially elongated and those of the inner 4–5 layers tangentially and obliquely oriented. The inner epidermis from the two valves produces inter-seed parenchymatous tissue (figure 26).

3.2c *Apical region*: In TS the ventral suture is slightly notched. In *P. cineraria* epidermis is one layered, thick-walled, tanniniferous. Hypodermal 4–5 layers are tanniniferous; the rest of the mesocarp is parenchymatous. Sutural vascular bundles are seen but not the endocarp (figure 31). The extreme apical region is parenchymatous with small intercellular spaces; tannin is present in some epidermal and hypodermal cells; lysiginous space is present in the central parenchyma (figure 30).

In *P. juliflora* the epidermis consists of thick-walled cells, heavily cuticularized on their outer walls. Hypodermal 2–3 layers are sclerenchymatous but without tannin cells. Vascular bundles are arranged in a ring, each with a sclerenchyma cap above it. The central region is the part of the endocarp but seed cavity is not distinct. The loose parenchyma filled with mucilage is between the vascular bundles and central sclerenchyma (figure 29). The extreme tip is parenchymatous bounded by epidermis. It contains a secretory sac-like structure and a small vascular bundle above the notch (figures 28, 33).

4. Discussion

Fahn and Zohary (1955) described 16 types and subtypes/forms of some types of the valvular structure in about 100 species of the Leguminosae and arranged them into 8 groups with reference to dehiscence emphasizing the structure of the sclerenchyma. These data clearly indicate the use of legumen structure as a taxonomic character to delimit taxa, but no types are specific for the subfamilies/families of the leguminosae. The legumen structure in *Calliandra* (Shah *et al* 1983) and *Neptunia* (Rangayya and Shah 1984 is *Acacia*, whereas it is *Trigonella* type in *Leucaena* (Shah and Rangayya 1983) and *Desmanthus* (Shah and Rangayya 1984). *Prosopis cineraria* shows *Trifolium* type of structure and *P. juliflora*, the *Cyanophyllum type*. The legumen structure thus can be used to separate the two species of *Prosopis* but there are some other differences of taxonomic significance and they are listed here.

Figures 28–33. **28, 29.** Transection of middle region. **30–33.** Transection of apical region. **28, 30, 31.** *P. cineraria*; **29, 32, 33.** *P. juliflora*. **28.** Secretory sac in mesocarp (× 229). **29.** Valvular region of mature pericarp (× 85). **30–33.** Apical region at different levels. **30, 31, 33.** × 85; **32** (× 150).

P. cineraria	*P. juliflora*
Stipe	
Hypodermis tanniniferous	Hypodermis not tanniniferous
Phloem fibres absent	Phloem fibres present
Lateral bundles not present at the junction of mature stipe with base of valves	Lateral bundles present
Mature pod	
Outer epidermal cells thickened on their outer walls	Outer epidermal cells thickened all round
Hypodermis tanniniferous	Hypodermis not tanniniferous
In valvular mesocarp hypodermal cells parenchymatous, tangentially elongated; other mesocarpic cells homogenous, except pitted cells scattered or in groups	In valvular mesocarp hypodermal cells sclerenchymatous. Innermost mesocarpic layers of cells elongated radially, filled with mucilage.
Valvular vascular bundles without sclerenchyma	Valvular vascular bundle with sclerenchyma cap
Endocarp collenchymatous of tangentially elongated cells	Endocarp sclerenchymatous, outer 2–4 layers radially elongated, inner 4–5 layers obliquely tangential
Apex	
Hypodermis tanniniferous	Hypodermis sclerenchymatous
Vascular bundles in two groups without sclerenchyma	Vascular bundles in a ring below hypodermis

References

Fahn A and Zohary M 1955 On the pericarpial study of the legumen, its evolution and relation to dehiscence; *Phytomorphology* **5** 99–111

Jensen W A 1962 *Botanical histochemistry*, (San Fransisco: Freeman)

Rangayya S and Shah G L 1984 Pericarpial structure in *Neptunia*; *Acta Bot. Indica* **12** 50–56

Shah G L, Rangayya S and Kothari I L 1983 Structure and development of pod of *Calliandra tweedii* Bth; *Flora* **173** 469–477

Shah G L and Rangayya S 1983 Structure of the pericarp of *Leucaena leucocephala* de Wit; *Feddes Rep.* **94** 649–656

Shah G L and Rangayya S 1984 Studies on the pericarp structure in *Desmanthus virgatus* Willd; *J. Pl. Anat. Morphol* **1** 31–38

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 11–31.
© Printed in India.

Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memoriam optimi Professoris Swamy

E GOVINDARAJALU
Department of Botany, Presidency College, Madras 600 005, India

MS received 2 June 1983; revised 6 September 1984

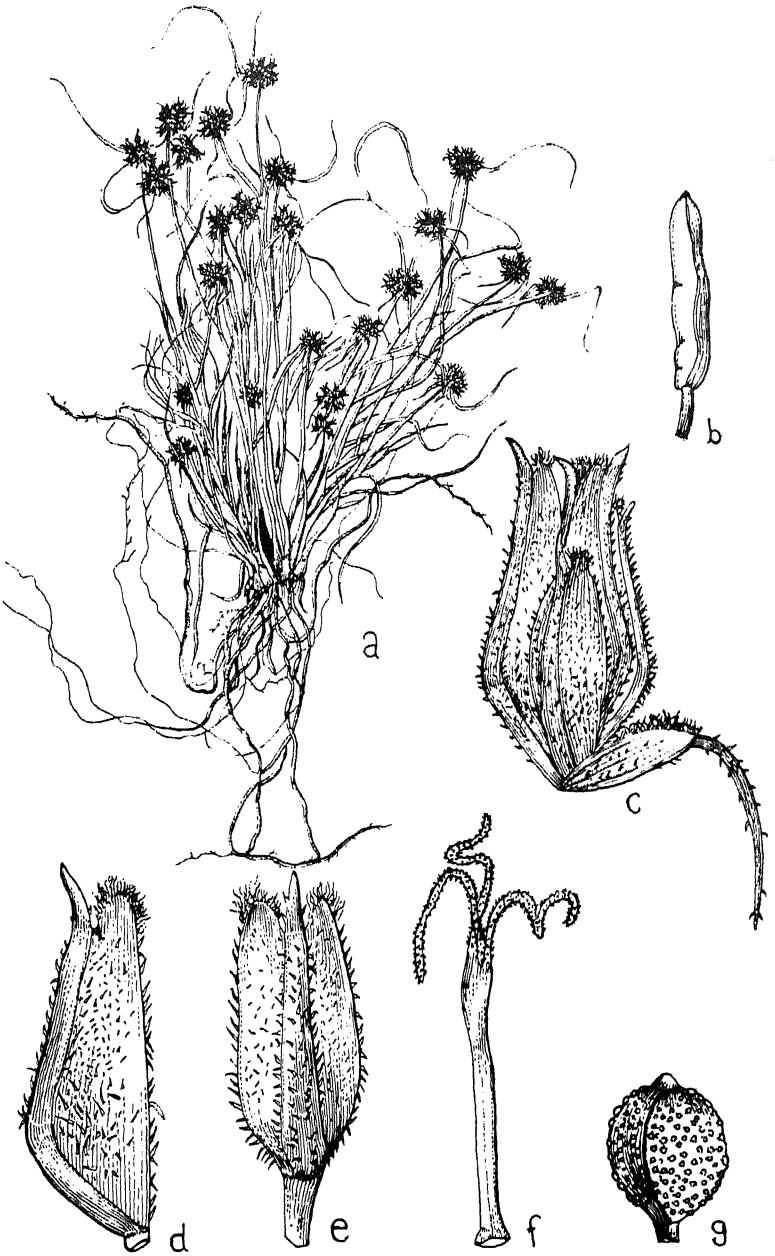
Abstract. Five new species belonging to *Bulbostylis, Eleocharis, Fimbristylis, Fuirena* an *Scleria* respectively have been proposed to immortalize late Prof. Dr Swamy for his valuabl botanical and nonbotanical contributions. The vegetative anatomy of these new species ha also been studied which shows an overall agreement with the general anatomical characters o each one of the genera besides the occurrence of a few characters specific for each species

Keywords. *Bulbostylis; Eleocharis; Fimbristylis; Fuirena; Scleria*; vegetative anatomy.

1. *Bulbostylis swamyii* Govind. sp. nov. (figure 1)

Annuales. Culmi caespitosi, setacei, glabri, leves, 3–10 cm × 0·3–0·5 mm. *Folia* varia longitudine, capillaria, scabrida per totum, 0·3–0·4 mm lata; vaginae cum mem branaceus 3–5 distincte nerviatis lateribus et cum densis longis capillis in distal marginis. *Inflorescentia* capitata, hemispherica, (3–) 5–10 (–12) spicularum consistens 5–8 (–10) mm lata. *Bracteae involucrales* 2–5, foliis consimiles, 2–3 quam inflorescentia longiores cum dilata scariosa margine sicut in vaginis, 1·5–3 cm longae, sursum et varie curvatae, scabridae ad marginem. *Spiculae* sessiles, urceolatae (ovatae), 6–8 florae, 3–5 × 1·5–1·8 (–2) mm. *Glumae* oblongae cum plus minusve laterales paralleliter, mem branaceae, valde bilobatae ad apicem, mucronatae, carinatae cum lateribus enervatis e dense scabrida per totum et cum longi capilli ad distalem marginem loborum cinnamomei fusci, 3–3·5 (inclusa mucrone) × 2·4–2·6 mm; carina latissima, valida uninervia, cinnamomea fusca, 0·8–1 mm lata; mucro recurvata, 0·5–0·7 mm longa *Stamina* 3; anthera oblonga linearia, aurantiaca vel porphyrea, minute calcarata ac basim et apiculata, 1·4–1·5 mm longa. *Stylus* tenuis, sparse ciliatus, 1·5–2 mm longus stigma 3, stylo multo breviora, papillata, 0·5–0·6 mm longa. *Nux* globosa, trigona dense granulata, albida vel straminea, minute stipitata, 0·7–0·8 × 0·5–0·6 mm.

Annuals. Culms caespitose, setaceous, glabrous, smooth, 3–10 cm × 0·3–0·5 mm. *Leaves* variable in length, capillary, scabrid throughout, 0·3–0·4 mm broad; sheaths with membranous 3–5 prominently nerved sides and with long hairs densely in the distal half margins. *Inflorescence* capitate, hemispherical consisting of (3–) 5–10 (–12) spikelets, 5–8 (–10) mm across. *Involucral bracts* 2–5, leaf-like, 2–3 times longer than inflorescence with dilated scarious hairy margin as in sheaths, 1·5–3 cm long, upwardly and variously curved, scabrid on margin. *Spikelets* sessile, urceolate (ovate), 6–8 flowered, 3–5 × 1·5–1·8 (–2) mm. *Glumes* oblong with almost parallel sides, membranous, deeply bilobed at apex, mucronate, keeled with nerveless sides and densely scabrid throughout and with long hairs at distal margin of lobes, cinnamomeous brown, 3–3·5 (incl.mucro) × 2·4–2·6 mm; keel very broad, strong, 1 nerved, cinnamomeous brown



Figures 1a–g. *Bulbostylis swamyii* Govind. *sp. nov.* **a.** habit × nat. size; **b.** stamen (× 20), **c.** spikelet (× 10), **d.** glume (× 18) (lateral view); **e.** glume (× 17) (spread out); **f.** style and stigma (× 27); **g.** nut (× 36) (all from Geetha 44, *type*).

0·8–1 mm broad; mucro recurved, 0·5–0·7 mm long. *Stamens* 3; anther oblong linear, orange yellow or reddish brown, minutely spurred at base and apiculate, 1·4–1·5 mm long. *Style* slender, sparsely hairy, 1·5–2 mm long; stigma 3, much shorter than style,

papillate, 0·5–0·6 mm long. *Nut* globose, trigonous, densely granulate, whitish or stramineous, minutely stipitate, 0·7–0·8 × 0·5–0·6 mm.

Related to *Bulbostylis barbata* (Rottb.) Clarke but differs in having leaves usually variable in length, urceolate few flowered spikelets, much longer involucral bracts, larger cinnamomeous brown deeply bilobed oblong glumes with almost parallel sides and with long hairs at distal margin of the lobes, longer mucro, 3 stamens, longer anthers and style, globose nuts with granulate surface.

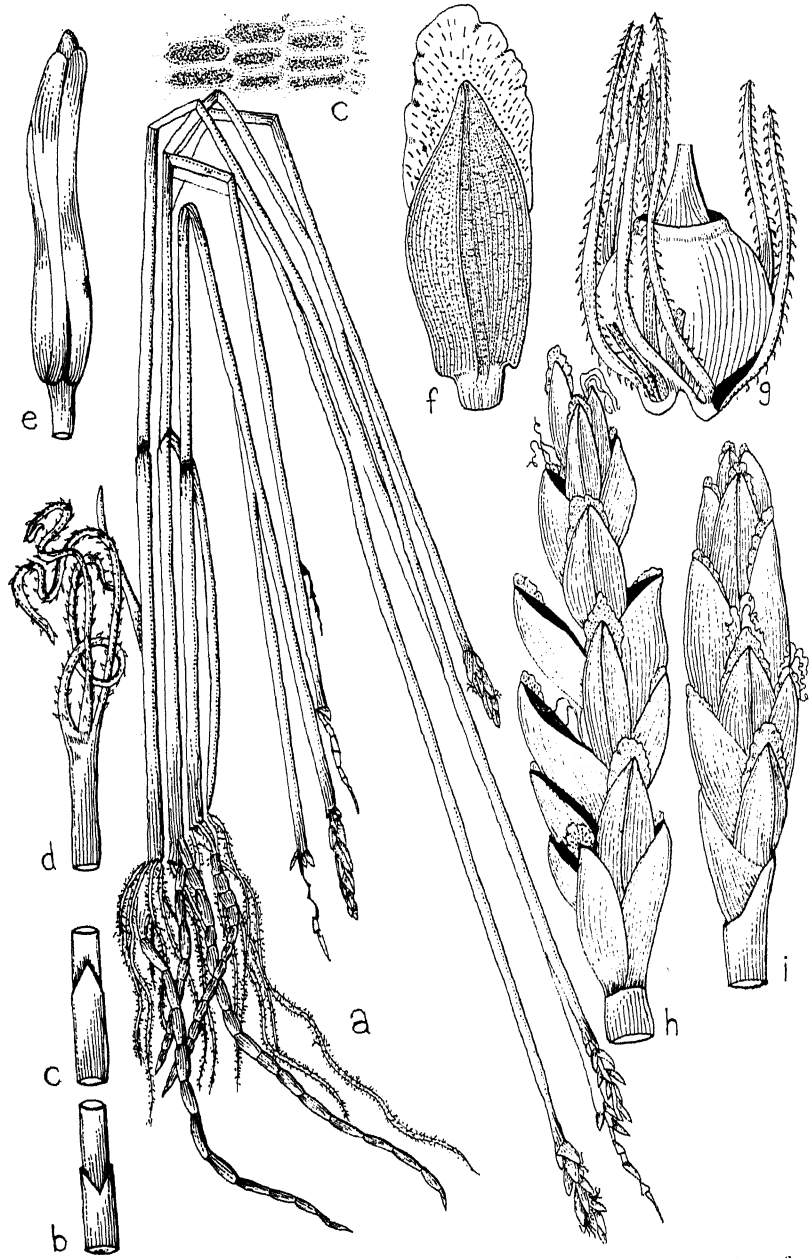
Geetha 44, Idinthakarai, Tirunelveli Dt., Tamilnadu, occurring in sandy places (*type*: CAL); *paratype*: Abdul Mallik, *s.n.*, Balasarasa 27, Chandra 31, Damodaran *s.n.*, Geetha and Akila 42, Kalairani 29, Rajeswari 21—all from the type locality (PCM).

Note: Specimens in dried condition assumes cinnamomeous brown color. The involucral bracts are usually recurved.

1. *Eleocharis swamyii* Govind. sp. nov. (figure 2)

Herba perennis cum rhizoma incrassatum, elongatum, oblique reptans. *Culmi* erecti, fasciculati, teretes per totum, laeves, non transverse septati, 30–60 cm × 2 mm. *Vaginae* membranaceae, plerumque sordide nigrae, nitidae, arcte amplectentes, oblique truncatae, acutae ad apicem, 10–15 cm longae. *Spiculae* cylindricae (cylindricae lanceolatae), teres postea angulares propter squarrosas glumas, culmo latiores, obtusae (acutae), multiflorae, pallide fuscae, 10–25 × 3·0–3·5 mm. *Glumae* firmae, cartilaginae, laxe imbricatae, adpressae, oblique erectae postea fiunt patescentes et convolutae ubi sicco, anguste ovatae vel ovato lanceolatae, subacutae vel acutae, multinerviae, sine manifesto medio nervo, late hyalinae in dimidio superiore, 3·5–4 × 2 mm; margine hyalinae, laceratae, tannin punctatae et striatae. *Setae* 7, inaequales, nux duplo longiores, retrorse totaliter scabridae, flavidae vel pallide fuscae. *Stamina* 2–3; anthera linearia, breviter apiculata, 1–1·5 mm longa. *Stylus* 2–2·5 mm longus; stigmata ramorum linearia, duplo quam stylo longiora, spiraliter, sparse pubescentia. *Nux* turgida, symmetrica, biconvexa, distincte cancellata, ecostata ad marginem, late orbiculata vel suborbiculata cum brevi collo manifeste marginata super collum, nitida, lutea vel testacea, 1·7–2 × 1·5 mm; cellulae epicarpicae largae, transverse elongatae hexagonales, in 20–25 regularibus seriebus praesentes in uno quoque; stylopodium deltoideum, planum, profunde fuscum, 1 cm longum.

Perennial herbs with thick obliquely creeping elongated rhizome. *Culms* erect, tufted, terete throughout, smooth, not transversely septate, 30–60 cm × 2 mm. *Sheaths* membranous, usually dirty black, shining, tightly clasping, obliquely truncate, acute at apex, 10–15 cm long. *Spikelets* cylindric (cylindric lanceolate), terete later becoming angular due to squarrose glumes, broader than culms, obtuse (acute), many flowered, pale brown, 10–25 × 3–3·5 mm. *Glumes* firm, cartilaginous, loosely imbricate, adpressed, obliquely erect, later becoming spreading and convolute when dry, narrowly ovate or ovate lanceolate, subacute or acute, many nerved without prominent mid nerve, broadly hyalined in upper half, 3·5–4 × 2 mm; hyaline margin lacerate, tannin punctate and striated. *Bristles* 7, unequal, 2 times longer than nut, retrorsely scabrous throughout, yellowish or light brown. *Stamens* 2–3; anther linear, shortly apiculate, 1–1·5 mm long. *Style* 2–2·5 mm long; stigmatic branches linear, 2 times longer than style, coiled, sparsely hairy. *Nut* turgid, symmetric, biconvex, distinctly cancellate,



Figures 2a–i. *Eleocharis swamyii* Govind. *sp. nov.* **a.** habit (× $\frac{1}{2}$) **b and c.** portion of culm showing sheath, diagrammatic; **d.** style and stigma (× 14); **e.** anther (× 37); **f.** glume (× 16); **g.** nut (× 13); **h.** spikelet (after anthesis) (× 5); **i.** spikelet (before anthesis) (× 5) (all from Govindarajalu, 8016, *type*).

ribless on margin, broadly orbicular or suborbicular with short neck, prominently rimmed above neck, shining, yellow or yellowish brown, 1·7–2 × 1·5 mm; epicarpic cells large, transversely elongated hexagonal, occurring in 20–25 regular rows on each face; stylopodium deltoid, flat, dark brown, 1 mm long.

Related to *Eleocharis variegata* (Poir.) Presl but differs in having erect culms always terete behind inflorescence, usually dirty black, shining acute sheaths, cylindric slightly shorter pale brown angular spikelets, more or less 4-ranked cartilaginous subcute glumes without ferrugineous margin and distinct midnerve, coiled unequal stigmatic branches nearly two times longer than style, shorter symmetric prominently longitudinally ridged nuts without marginal ribs and with 20–25 rows of transversely elongated hexagonal cells on each face, 7 bristles being two times longer than nut and retrorsely scabrous up to base.

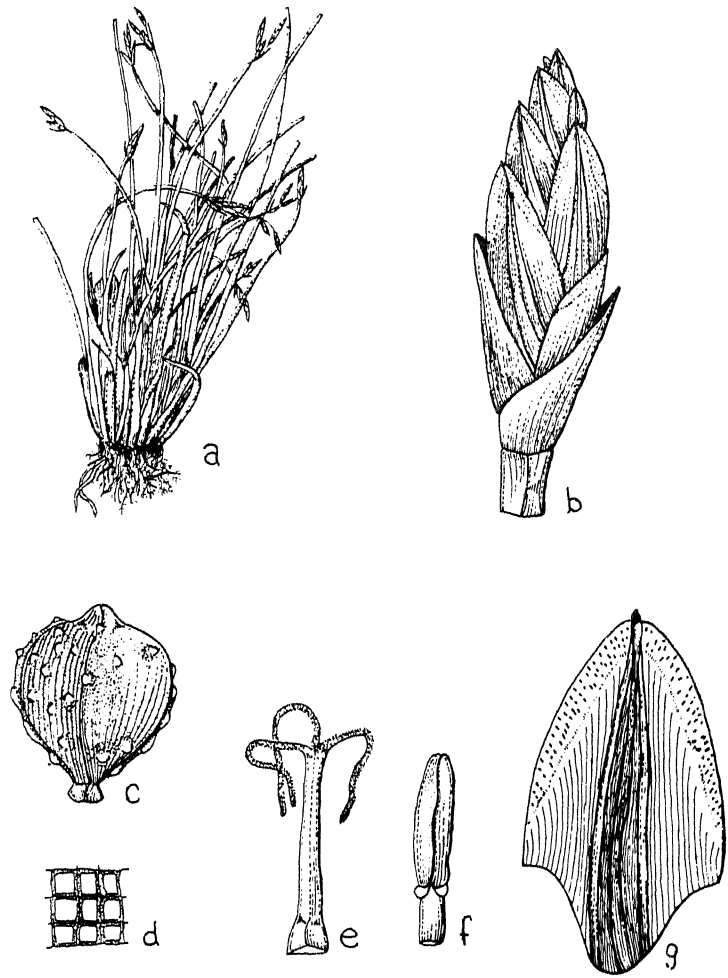
Govindarajalu 8016, Kambakkam, Andhra Pradesh (*type*: CAL) *isotype*: 8016 A–I: 8016 A, MH; 8016 B, BLAT; 8016 C, DD; 8016 D, BSI; 8016 E–I, PCM; *paratype*: Ernest Thayasingh 9547, Vellachithode, Kodhaiyar, Kanyakumari Dt., Tamilnadu (PCM).

Note: (i) Rather commonly found from mean sea level up to 500–600 m. (ii) The broad lacerate hyaline margin present in the distal half of glumes ultimately become deliquescent. (iii) The glumes become convolute at later stages. (iv) The whole plant becomes shiny after drying. (v) The individual glumes become separated and effuse in the mature spikelets.

23. *Fimbristylis swamyii* Govind. sp. nov. (figure 3)

Annuales. Culmi tenui, setacei, dense caespitosi, 4–5 goni, glabri, laeves, costati, sulcati, 4–8 cm × 0·4–0·6 mm. *Folia* culmo breviora, capillacea, glabra, 3–5 nervia, omnino margine laevigata, ligulata, *ca* 0·5 (–1) mm lata; vaginae membranaceae, glabrae, retro rotundatae. *Inflorescentia* semper simplex, compacta, 3–5 spiculis consistens, 5–8 × 3–4 mm. *Bracteae involucrales* 2–3, multum inflorescentia breviora, 3–4 mm longae. *Radii* 4–5 goni, laeves, portantes 1 (–3) spiculas. *Spiculae* solitariae (–3), ellipticae ovatae, angulares, acutae, laxe pauciflorae, pallide cinnamomeae fuscae, 3–4 × 1–1·5 mm. *Rhachilla* alata, excavata. *Glumae* spiraliter, chartaceae, oblongae ovatae cum distincta hyaline margine, mucronulatae, carinatae, glabrae cum lateribus enervatis, 2–2·2 × 1·2–1·4 mm; carina cum 3 nervis; nervi in mucronem excurrentes. *Stamina* 3; anthera linearis oblonga, minute apiculata, *ca* 0·5 mm longa. *Stylus* triquetrus cum basi pyramidali, glaber 0·6–0·8 mm longus; stigma 3, glabra, plus minusve stylo aequilonga. *Nux* late obovoidea, trigona, stipitata, umbonulata, pallide fusca, tuberculata cum pluricellulares auctus, 0·8–0·9 (incl. stipe) × 0·7 mm; cellulae epicarpicae isodiametricae in 10–12 (–15) regularibus seriebus in una quoque facie dispositae; stipe 0·1 mm longa.

Annuals. Culms slender, setaceous, densely caespitose, 4–5 gonus, glabrous, smooth, ribbed, sulcate, 4–8 cm × 0·4–0·6 mm. *Leaves* shorter than culms, capillary, glabrous, 3–5 nerved, smooth margined throughout, ligulate, *ca* 0·5 (–1) mm broad; sheaths membranaceous, glabrous, rounded at back. *Inflorescence* always simple, compact, consisting of 3–5 spikelets, 5–8 × 3–4 mm long. *Rays* 4–5 gonus, smooth bearing 1 (–3) spikelets. *Spikelets* solitary (–3), elliptic ovate, angular, acute, loosely few flowered, pale cinnamomeous brown, 3–4 × 1–1·5 mm. *Rhachilla* winged, excavated. *Glumes* spiral, chartaceus, oblong ovate with distinct hyaline margin, mucronulate, keeled, glabrous with nerveless sides, 2–2·2 × 1·2–1·4 mm; keel 3 nerved; nerves excurrent into mucro. *Stamens* 3; anther linear oblong, minutely apiculate, *ca* 0·5 mm long. *Style* triquetrus with pyramidal base, glabrous, 0·6–0·8 mm long; stigma 3, glabrous, almost as long as



Figures 3a–g. *Fimbristylis swamyii* Govind. *sp. nov.* **a.** habit (×½); **b.** spikelet (× 15); **c.** nut (× 33); **d.** epicarpic cells, diagrammatic; **e.** style and stigma (× 36); **f.** anther (× 45); **g.** glume (× 28); (all from Govindarajalu, 14816 C, *type*).

style. *Nut* broadly obovoid, trigonus, stipitate, umbonulate, pale brown, tuberculate with pluricellular outgrowths, 0·8–0·9 (incl.stipe) × 0·7 mm; epicarpic cells isodiametric arranged in 10–12 (–15) regular rows on each face; stipe 0·1 mm long.

Related to *Fimbristylis capilliculmis* Ohwi but differs in having shorter 4–5 gonus culms, smooth margined shorter leaves, always simple compact smaller inflorescence containing lesser number of spikelets, involucral bracts much shorter than inflorescence, 4–5 gonus rays, elliptic ovate smaller spikelets, chartaceus ovate glumes with distinct hyaline margin, always 3 stamens, shorter style, stigma as long as style, tuberculate nuts with pluricellular outgrowths and quadrate epicarpic cells arranged in distinct rows.

Govindarajalu 14816 C, Cliff View Forests, Nelliampathy, Pothundy, Kerala State (*type*: CAL); *isotype*: 14816 C, MH; BSI; BLAT; DD; *paratype*: Govindarajalu 14391, 14394, Thangaikal Mottai, Highwavys Mts., Madurai Dt., Tamilnadu, (PCM).

Note: (i) Common in grasslands at 1000–1500 m. (ii) Straw like texture coupled with reddish brown color and much shorter bracts seem to be the characteristic features of this species.

1. *Fuirena swamyii* Govind. sp. nov. (figure 4)

Herba perennis, rhizomata. *Rhizoma* brevis, oblique reptans, lignea cum luridis ve nigris, multinervis, ovatis, lanceolatis squamis obtecta. *Culmi* triquetri, fasciculati, plu minusve erecti, leviter gracili, glabri per totum, non incrassati ad basim, 20–45 cm × 2–2·5 mm. *Folia* rigida, oblique erecta, lanceolata, acuminata, carinata, 10–13 nervia sulcata adaxiali cum incrassato recurvato margine, quoque internodis longiora, 6–8 (–9) cm × 3–4 mm; carina et adaxiales sulcus cum trichome seriebus; vaginae internodi breviorae, multinerviae cum ore truncato ciliato. *Inflorescentia* terminalis et sub terminalis racemi portati pedunculis pubescentibus, 1–2 cm lati, 3–10 spicularun consistens; bracteae inferiorae foliis consimilis, plerumque inflorescentia longiorae oblique erectae, vaginans; bracteae superiores ovate lanceolatae (setaceae), erectae hirsutae ad marginem et basim, inflorescentia aequilongae vel breviores, non vaginans *Spiculae* ovoidae, acutae, canae vel nigrae fuscae, 6–9 × 2–3 mm. *Glumae* ovatae rotundatae ad apicem, dense hirsutae, carinatae, *ca* 3 indistinctae laterales nervia quoque in dimidis, abundater tannin punctatae, aristatae, 3–3·2 (incl. arista × 1·4–1·5 mm; carina cum 3 nervis; nervi in aristam excurrentes; arista erecta aliquantum glabra, 0·6–0·7 mm longa. *Setae* 6, bene exultae, biseriatae, fuscae, retrorsc scabridae; sepaline setae nuci aequilongae, *ca* 1·5 mm longae; petaline setae nuc longiorae, *ca* 2 mm longae; squamae 0. *Stamina* 3; anthera linearis oblonga, candida ve lutea, minute apiculata, 0·6–0·8 mm longa. *Nux* late elliptica vel plus minusve obovata triquetra, tricostulata, laevis, nitida, glandacea fusca, 1·5 (incl. stipes et rostrum × 0·6–0·7 mm; cellulae epicarpicae minutae, isodiametricae, indistinctae; rostrum pyramidalis, laevis cum margine undulata, *ca* 0·3 mm longus; stipes 0·4 mm longus

Perennial herbs, rhizomatous. *Rhizome* short, obliquely creeping, woody covered with dirty brown or black multinerved ovate lanceolate scales. *Culms* triquetrus, tufted, more or less erect, rather slender, glabrous throughout, not swollen at base, 20–45 cm × 2–2·5 mm. *Leaves* rigid, obliquely erect, lanceolate, acuminate, keeled, 10–13 nerved adaxially grooved with thickened recurved margin, longer than each internode, 6–8 (–9 cm × 3–4 mm; keel and adaxial grooves with rows of hairs; sheaths shorter than internodes, many nerved with truncate hairy mouth. *Inflorescence* terminal and subterminal clusters borne by pubescent peduncles, 1–2 cm broad consisting of 3–10 spikelets; lower bracts similar to leaves usually longer than inflorescence, obliquely erect, sheathing; upper bracts ovate lanceolate (setaceous), erect, hirsute at margin and base, as long as or shorter than inflorescence, not sheathing. *Spikelets* ovoid, acute greyish or blackish brown, 6–9 × 2–3 mm. *Glumes* ovate, rounded at apex, densely hirsute, keeled with *ca* 3 indistinct lateral nerves in each half, abundantly tannin punctate, aristate, 3·0–3·2 (incl. arista) × 1·4–1·5 mm; keel 3 nerved; nerves excurrent into arista; arista erect, somewhat glabrous, 0·6–0·7 mm long. *Bristles* 6, well developed biseriate, brown, retrorsely scabrid; sepaline bristles as long as nut, *ca* 1·5 mm long petaline bristles longer than nut, *ca* 2 mm long; scales 0. *Stamens* 3; anther linear oblong pale white or yellow, minutely apiculate, 0·6–0·8 mm long. *Nut* broadly elliptic or more or less obovate, triquetrus, tricostulate, smooth, shining, yellowish-brown, 1·5 (incl

Figures 4a–g. *Fuirena swamyii* Govind. *sp. nov.* **a.** habit (×$\frac{1}{2}$), **b and c.** adaxial and abaxial surfaces of lamina showing hairs in the median regions and keel, diagrammatic; **d.** anther (× 20); **e.** nut with bristles (× 30); **f.** spikelet (× 8); **g.** glume (× 20); (all from Govindarajalu, 12252, *type*).

stipe and beak) × 0·6–0·7 mm; epicarpic cells minute, isodiametric, indistinct; beak pyramidal, smooth with more or less undulate margin, *ca* 0·3 mm long; stipe 0·4 mm long.

Related to *Fuirena pubescens* Kunth but differs in having glabrous culms, rows of hairs on the keel and adaxial grooves of leaves, 6 well-developed retrorsely scabrid

brown hypogynous bristles, sepaline bristles as long as and the petaline ones longe than nut, distinctly stalked yellowish brown or brown nuts with stalk more or les quarter the length of the nuts and minute indistinct isodiametric epicarpic cell arranged in rows.

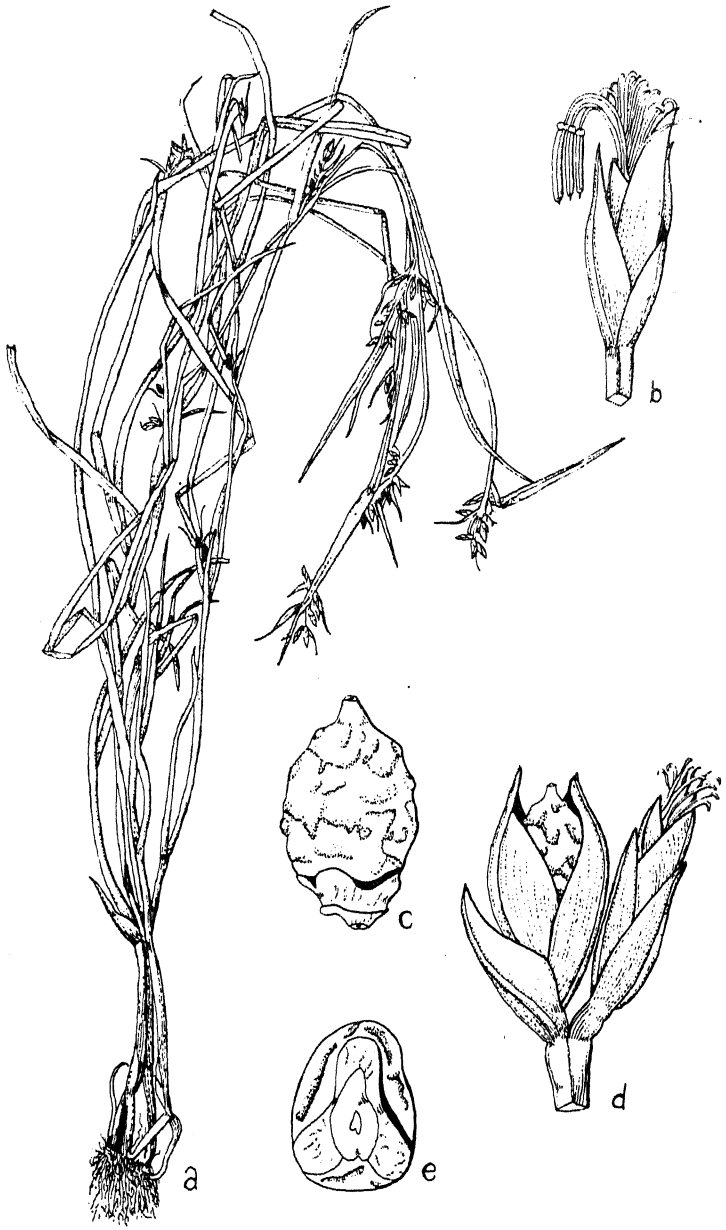
Govindarajalu 12252, Ervangalar 17th field, Highways Mts., Madurai Dt. Tamilnadu (*type*: CAL); *isotype*: 12252 A, MH; 12252 B, BSI; 12252 C, BLAT; 12252 D, DI 12252 E–I, (PCM).

Note: (i) Common in marshy places in grass lands. (ii) Characterized by greyish blacl spikelets and the presence of hairs over the keel and the median adaxial grooves of th leaves.

3. *Scleria swamyii* Govind. sp. nov. (figure 5)

Herba perennis, glabra, levis. *Culmi* leviter tenues, fasciculati, erecti, acute triquetri cun plana facie, retrorso scabridi, 40–70 cm × 2–2·5 mm. *Folia* gradatim contracta i summa acuta, glabra cum margine serrulata in superiore 1/3 folii, 3–4 mm lata distributa in intervallis fere equis; vaginae angustae, non alatae, leves, aliquan pubescentes vel pilosa in dimidio superiore; vaginae infimae purpureae fusca puberulentae; contraligula multum breviora, lunata, crassa, latiore quam longiora ciliata cum margine scariosa. *Inflorescentia* leviter lata, elongata, plerumque consisten unius paniculi (interdum 1 lateralis addita), 2–3 × 1–1·5 cm, paniculis plus minusv laxis; pedunculis solitari (binatis), plerumque breviores, pubescentes ad apicem bracteae primarie plus minusve foliis consimiles, inflorescentia quam longiora secondari setacei. *Spiculae* unisexuales, 2 (–3) in fasciculati, purpureis fuscis; spicula masculae breviter pedunculatae, obliquae oblongae ovatae, 4–5 mm longae; stamina 3 antherae linearia, *ca* 1 mm longae, apiculatae, calcaratae ad basim; spiculae femineae ellipticae ovatae, 2·5–3 mm longae; glumae 3, late ovatae, acutae vel apiculatae, glabrae *Discus* distincte tenuiter trilobatus; lobi oblongi, rigidi, rotundati, plus minusve nuc adpressi, integris, pallidi, striolati, 0·5–0·7 mm longi cum sinis latis. *Nux* alabastrina nitida, glumae aequilonga vel leviter subglobosa, glabra, manifeste rostrata, teretis rugulosa transverse undulata, ferruginea, tenaciora juga cum 3 depressionibus ac basim, 2·5–2·6 (incl.rostro) × 2 mm; rostrum *ca* 0·2 mm longum.

Perennial herbs, glabrous, smooth. *Culms* rather slender, fascicled, erect, acutely triquetrus with flat faces, retrorsely scabrid, 40–70 cm × 2–2·5 mm. *Leaves* gradually narrowed into acute tip, glabrous with serrulate margin in upper 1/3 of leaf, 3–4 mm broad, distributed almost at equal intervals; sheaths narrow, wingless, smooth sometimes puberulous or pilose in upper half; lowermost sheaths purplish brown puberulous; contraligule very short, lunate, thick, broader than long, ciliate with scarious margin. *Inflorescence* rather broad, elongate consisting of usually single panicle (sometimes 1 lateral added), 2–3 × 1–1·5 cm; panicle somewhat lax; peduncles solitary (binate), usually short, hairy at top; primary bract more or less similar to leaves longer than inflorescence, secondary ones setaceous. *Spikelets* unisexual, in clusters of 2 (–3), purplish brown; male spikelets shortly peduncled, oblique, oblong ovate, 4–5 mm long; stamens 3; anther linear, *ca* 1 mm long, apiculate, spurred at base; female spikelets elliptic ovate, 2·5–3 mm long; glumes 3, broadly ovate, acute or apiculate, glabrous. *Disk* distinctly shallowly 3 lobed; lobes oblong, rigid, rounded, more or less adpressed to nut entire, pale, striolate, 0·5–0·7 mm long with broad sinuses. *Nut* marble white, shining, a



Figures 5a–e. *Scleria swamyii* Govind. *sp. nov.* **a.** habit (× ½); **b.** male spikelet (× 9); **c.** nut (× 15); **d.** female and lateral male spikelet (× 15); **e.** base of nut showing disc (× 10) (all from Govindarajalu, 12515, *type*).

long as or shorter than glumes, subglobose, glabrous, prominently rostrate, rugulose by transverse undulate ferrugineous viscid ridges with 3 depressions at base, 2·5–2·6 (incl.rostrum) × 2·0 mm; rostrum *ca* 0·2 mm long.

Related to *Scleria oblata* Blake but differs in the following respects: culms retrorsely scabrid; leaves narrower, distributed more or less at equal intervals; inflorescence

shorter, narrower and lax; anthers shorter; female spikelets shorter with glabrous glumes; disk lobes oblong, shorter, rounded at apex with broad sinuses; nuts larger than glumes, subglobose, prominently rostrate, rugulose by transverse undulate ferrugineous viscid ridges with 3 depressions at base.

Govindarajalu 12515, on the way to Italiyar to Anamaliyar, Valparai, Coimbatore Dt., Tamilnadu (*type*: CAL); *isotype*: 12515 A, MH; 12515 B, BLAT; 12515 C, PCM.

Note: (i) Rare in open wet grassy places occurring at 1000–1250 m. (ii) Leaves distributed throughout the plant almost at equal intervals.

Vegetative anatomy

For methods and other information, see Govindarajalu (1966, 1968 a,b, 1975) and Metcalfe (1971). The descriptive terms proposed by Metcalfe and Gregory (1964) are followed here.

Bulbostylis swamyii

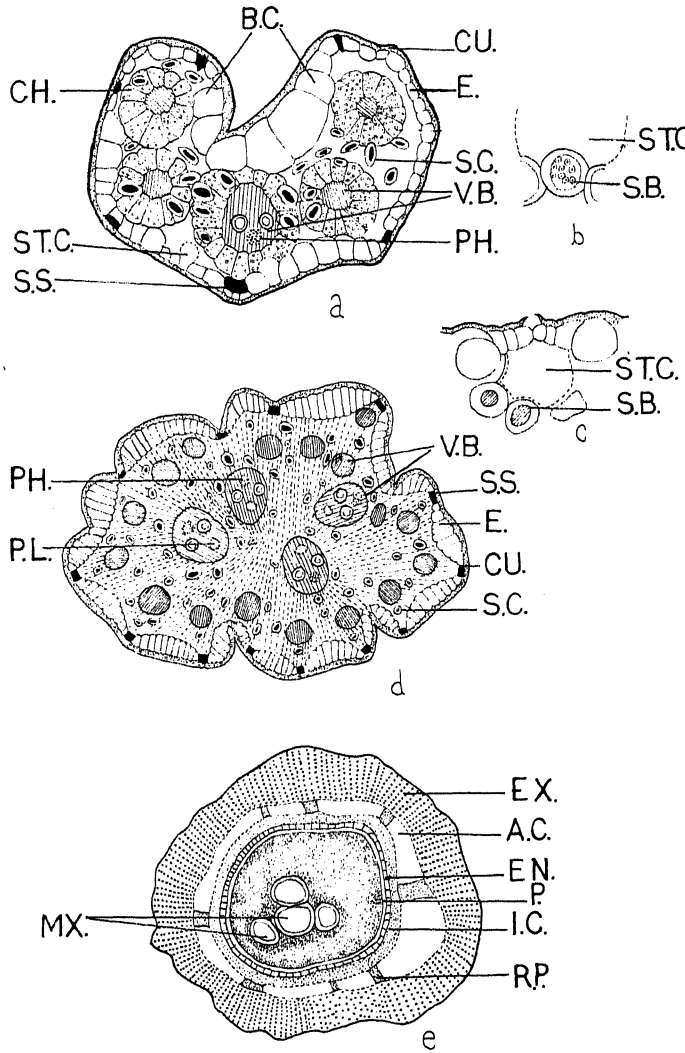
Leaf—*Abaxial surface*: Intercostal cells axially elongated; cell walls thin, almost smooth. Stomata (length 27–56 μm; width 21·6–23·4 μm), more or less circular or elliptic; subsidiary cells low dome-shaped; interstomatal cells moderately long with concave ends. Silica-cells elongated, narrow, each cell containing 2–3 silica-bodies without satellites. Prickle hair short (length 90–102·5 μm), excessively thick-walled, pointed with or without narrow lumen, bulbous based, curved or erect commonly present on surface and margin.

Adaxial surface: Cells broad, hexagonal; cell walls thin, smooth; stomata and silica-cells absent.

T. S. lamina: Thickly V-shaped as in figure 6a. Width of lamina examined *ca* 0·3 mm. Epidermis: abaxial epidermal cells inflated but *ca* 6 cells in adaxial concavity much larger than the remainder thus resembling bulliform cells. Cuticle thicker on the adaxial surface than on the abaxial. Sclerenchyma: abaxial (height 13·5 μm; width 22·5 μm) squarrish, subtending each vb; median vb with adaxial girder, see abaxial strand; adaxial marginal strand more or less triangular (height 18 μm; width 22·5 μm). Air-cavities not distinct; substomatal cavities larger than the size of epidermal cells. Chlorenchyma radiating separated by translucent cells; vb's 5 forming an arc; median vb larger than the remainder; lateral vb's circular in outline while the median ones are oval shaped; median vb (type III A) and lateral vb's (type I; figure 6a); metaxylem vessel element of median vb (18 μm in diameter); metaphloem of 'intermediate type'. Bundle sheath seemingly 2 layered, both complete; os fibrous; IS parenchymatous not distinct. Silica-bodies smooth more or less spherical, present in the inner bordering cells of substomatal cavity (figure 6c). Secretory cells large, abundant.

Culm: *Epidermis, surface view*: Cells elongated, variable in size and shape; cell walls moderately thick, smooth. Silica-cells short, rather broad, occurring in 2 rows, each cell containing 1–2 (–3) nodulose bodies. For other details, see leaf.

T. S. Culm: Outline irregular with 3 narrow deep furrows and *ca* 3 wider shallow furrows; portions between furrows representing 3–5 minor rounded ribs (figure 6d). Diameter of culm examined 0·5–0·6 mm. Central ground tissue consisting of thin-walled, large parenchyma cells without intercellular spaces. Cuticle rather thick.



Figures 6a–e. *Bulbostylis swamyii* Govind. **a.** transection of lamina (× 133); **b.** transection of substomatal chamber in culm (× 340); **c.** transection of stoma and substomatal chamber in lamina (× 340); **d.** transection of culm (× 135); **e.** transection of root (× 133) (from Geetha, 44, *type*).

Epidermal cells inflated, radially elongated and arranged in regular groups (figure 6d) and their continuity being interrupted by sclerenchyma. Air-cavities present as substomatal ones, see lamina; inner bordering cells of substomatal cavity containing silica particles (figure 6b). Assimilatory tissue: chlorenchyma radiating, completely so in small vb's and incompletely or crescentiform like in large vb's. Sclerenchyma strands *ca* 17 (–20) usually squarrish (height and width 22·5 μm); few triangular (height 31·5 μm; width 22·5 μm). Vb's *ca* 20 occurring in more or less 2 rings, outer ring consisting of *ca* 13 vb's (type I), small, circular, inner ring of 4 vb's large, oval shaped (type III B), the latter containing protoxylem lacunae; metaxylem elements (13·5–18 μm in diameter);

metaphloem of 'regular type'. Bundle sheath 2 layered, os fibrous, is parenchymatous, incomplete in large vb's; is indistinct in small vb's. Secretory cells as in lamina.

T. S. Root: Diameter of root examined *ca* 0·3 mm. Exodermis of 1–2 layers of large variable cells. Cortex recognizable into 2 zones, outer lacunose but connected with exodermis at few places by short radiating rows of cells; inner of 2–3 layers of small rather thick-walled cells, compactly arranged (figure 6c). Endodermis very conspicuous made up of radially elongated cells having uniform thickening all around and reduced lumina. Pericycle distinct with rather thick-walled cells. Metaxylem elements *ca* 4, large, thin-walled, central (figure 6e), each somewhat oval-shaped (27–36 μm in diameter). Metaphloem and protoxylem not distinct. Central ground tissue compact consisting of rather thick-walled cells (Govindarajalu 1966).

Eleocharis swamyii

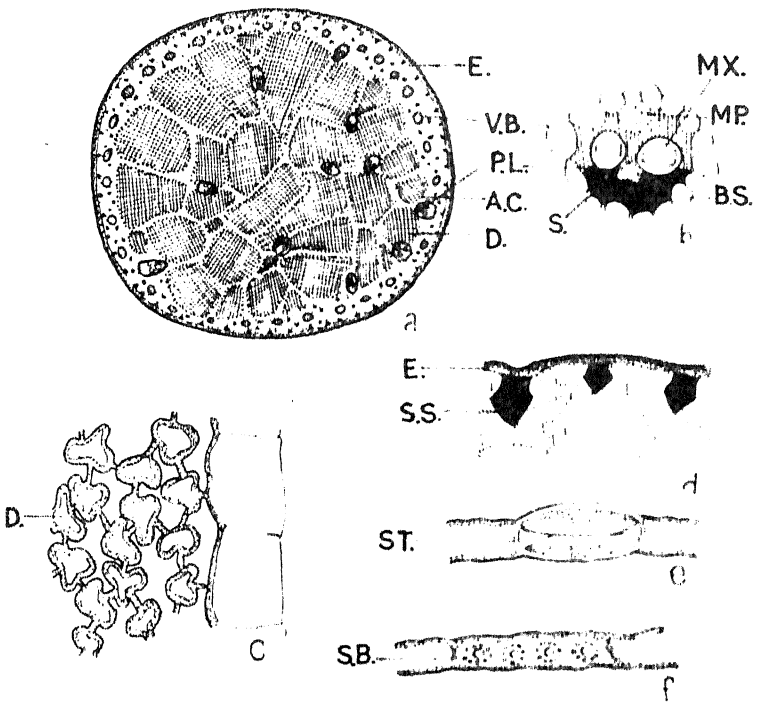
Sheath-*Abaxial surface*: Cell walls smooth. Silica-cells not observed. For other details, see culm.

T. S. Sheath: Epidermal cells moderately large, radially elongated, thick-walled. Cuticle thin. Abaxial surface slightly ribbed. Sclerenchyma strands (height 14·6 μm; width 14·6–21·9 μm), very minute consisting of 6–8 cells, inversely securiform. Air-cavities as many as vb's almost regularly alternating with them, each air-cavity containing stellate fairly thick-walled parenchyma cells enclosing usually trilobed interspaces; air-cavities delimited by a single row of large parenchyma cells. Vb's *ca* 27 in number being large and small, the former of type III A and the latter type I; metaxylem vessel elements (22·2 μm in diameter), circular in outline; metaphloem of 'regular type'. Bundle sheath single, complete, parenchymatous. Secretory cells common.

Culm—*Epidermis, surface view:* Cells axially elongated, narrow; cell walls thin, moderately sinuous. Stomata (length 40·5–43·4 μm; width 17·4 μm), axially elongated, oblong elliptic, narrow; subsidiary cells parallel sided (figure 7e); interstomatal cells elongated with concave ends. Silica-cells long, narrow, thin-walled each cell containing 4–5 silica-bodies with satellites occurring in a single discontinuous row (figure 7f).

T. S. Culm: Outline somewhat circular (figure 7a). Diameter of the culm examined *ca* 2·6 mm. Epidermal cells relatively small, thick-walled. Cuticle thin. Assimilatory tissue: 2–3 layers of radially elongated chlorenchyma cells situated next to epidermis. Air-cavities *ca* 30 in number, variable in size and shape (figure 7a), each traversed by diaphragms of thick-walled stellate parenchyma; see sheath for other details. Vb's *ca* 36 in number arranged in peripheral and central systems; large vb's (type: III A) with protoxylem lacunae and small vb's (type I) (figure 7b); peripheral system consisting of *ca* 30 vb's out of which few large vb's project little towards the centre; the central vb's *ca* 6 scattered in net tissue; metaxylem vessel elements (23·2 μm in diameter), circular in outline, metaphloem of 'regular type'. Bundle sheath single, complete with large parenchymatous cells in all vb's; crescentiform cap of 2–3 layered sclerenchyma present at xylem poles of all vb's. Sclerenchyma strands and secretory cells, see sheath.

T. S. Root: Diameter of specimen examined *ca* 1 mm. Exodermis: cells isodiametric, thick-walled. Root hairs abundant. Cortex recognizable into outer and inner zones, the former narrow consisting of 3–4 layers of compactly arranged cells similar to those of exodermis and the latter broad, lacunose; air-cavities concentrically arranged and



Figures 7a f. *Eleocharis swamyii* Govind. **a.** transection of culm (× 22), **b.** transection peripheral vascular bundle (× 180); **c.** transection of diaphragm cells (× 380); **d.** sectional vie of sclerenchyma strands (× 380); **e.** surface view of stoma (× 590), **f.** surface view of silica-ce (× 590); (from Govindarajalu, 8016, *type*)

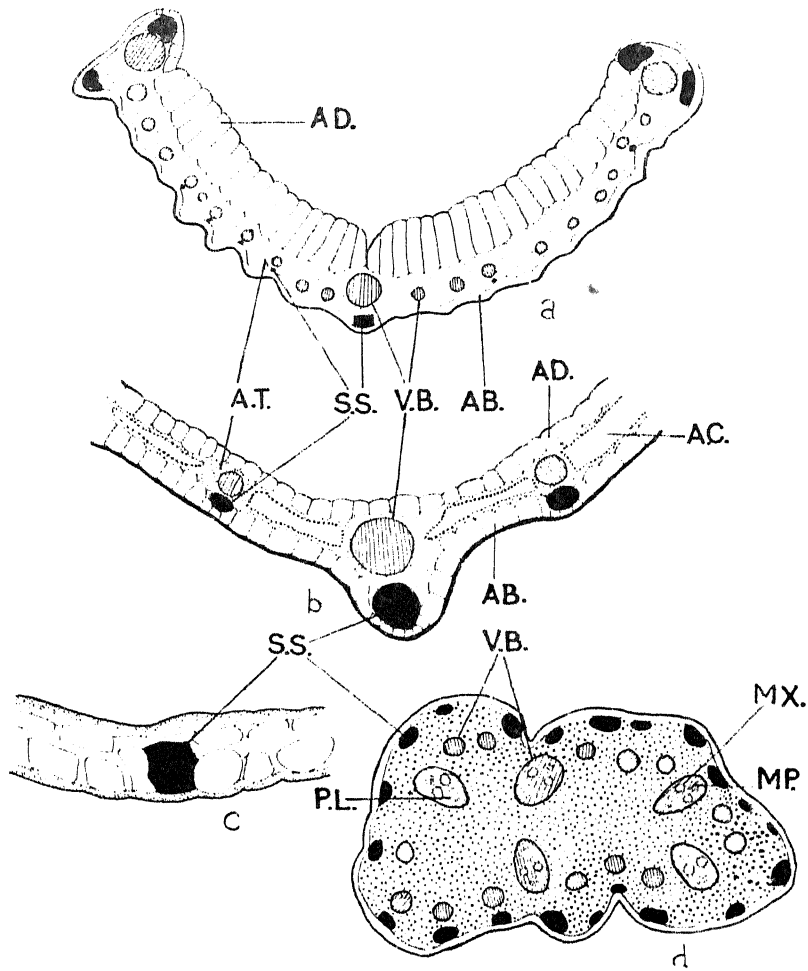
separated from one another by radiating rows of parenchyma cells. Endodermis: cel radially elongated with uniform thickening all around and broad lumina. Pericycle n distinct. Xylem represented by 9 metaxylem units, each oval-circular in outlin elements (22·5–27 μm in diameter). Metaphloem rather distinct consisting of 2–3 lar sieve tube elements. Central ground tissue sclerenchymatous (Govindarajalu 1975

Fimbristylis swamyii

Leaf—*Abaxial surface*: Intercostal cells variable in size; cell walls thin, sinuou Stomata (length 27–31·5 μm; width 27 μm), elliptic – suborbicular in outline; subsidia cells low dome-shaped; interstomatal cells short with concave ends. Silica-cel occurring in 2–3 discontinuous rows, each cell elongated, narrow containing 2–4 cor shaped silica-bodies surrounded by satellites.

Adaxial surface: Cells axially elongated, broad; cell walls, see abaxial surface. Stomat and silica-cells absent.

T. S. lamina: Thinly crescentiform, abaxially many ribbed; keel indistinct (figure 8a Width of specimen examined (2·6 mm). Margins unequal. Adaxial epidermal cells ver inflated and much larger than those of the abaxial epidermis with the latter varying i size and those over sclerenchyma strands much smaller than the remainder. Stomat restricted to each abaxial rib. Hypodermis, see mesophyll. Sclerenchyma: abaxial an



Figures 8a–d. *Fimbristylis swamyii* Govind. **a.** transection of lamina, diagrammatic; **b.** portion of sheath in transection (× 133); **c.** transection of sheath margin (× 380); **d.** transection of culm (× 93) (from Govindarajalu, 14816 C, *type*).

adaxial strands of marginal vb's pulviniform (height 13·5–31·5 μm; width 27–45 μm); keel vb with rectangular strand (figure 8a; height 27 μm; width 36 μm); few small vb's accompanied by rounded strands (height and width 13·5 μm); however strands tending to become girders. Mesophyll not well differentiated; chlorenchyma radiate and confined to abaxial one third of lamina. Air-cavities present as small substomatal cavities. Bulliform cells of typical ones wanting (figure 8a). Vb's *ca* 22, large and small not regularly alternating with each other forming an abaxial arc out of which 3 are prominently larger (type III A); metaxylem vessel elements (13·5 μm in diameter). Bundle sheaths 3 layered, complete; OS and IS parenchymatous, MS fibrous. Secretory cells less common.

Culm—*Epidermis, surface view*: Cells axially elongated; cell walls rather thick, sinuous. Stomata (length 31·5–36 μm; width 22·5–27 μm), elliptic oblong, see abaxial surface.

Silica-cells occurring in 1–2 discontinuous rows, each cell elongated, narrow, containing (4–) 5–6 cone shaped silica-bodies with satellites.

T. S. Culm: Outline as in figure 8d, trapezoidal with a few conspicuous furrows. Long diameter of culm examined *ca* 0·6 mm. Cuticle smooth, thick. Epidermal cells large, variable in size. Air-cavities narrow, represented by substomatal chambers. Assimilatory tissue 3–4 layered, not radiate. Sclerenchyma pulviniform (height 22·5–36 μm; width 36–45 μm). Vb's *ca* 19 present more or less in a single circle and embedded in chlorenchyma; vb's of two sizes; 5 large (type III A); 14 small (type I) the large vb's slightly pe[illegible]rating into the central ground tissue; metaxylem vessel elements (13·5–18 μm in diame[illegible]); large vb's containing protoxylem lacunae. Metaphloem of 'intermediate type'. Bundle sheaths 2 layered, complete in all vb's; os parenchymatous; is fibrous. Central ground tissue of very inflated polygonal cells. Secretory cells less common.

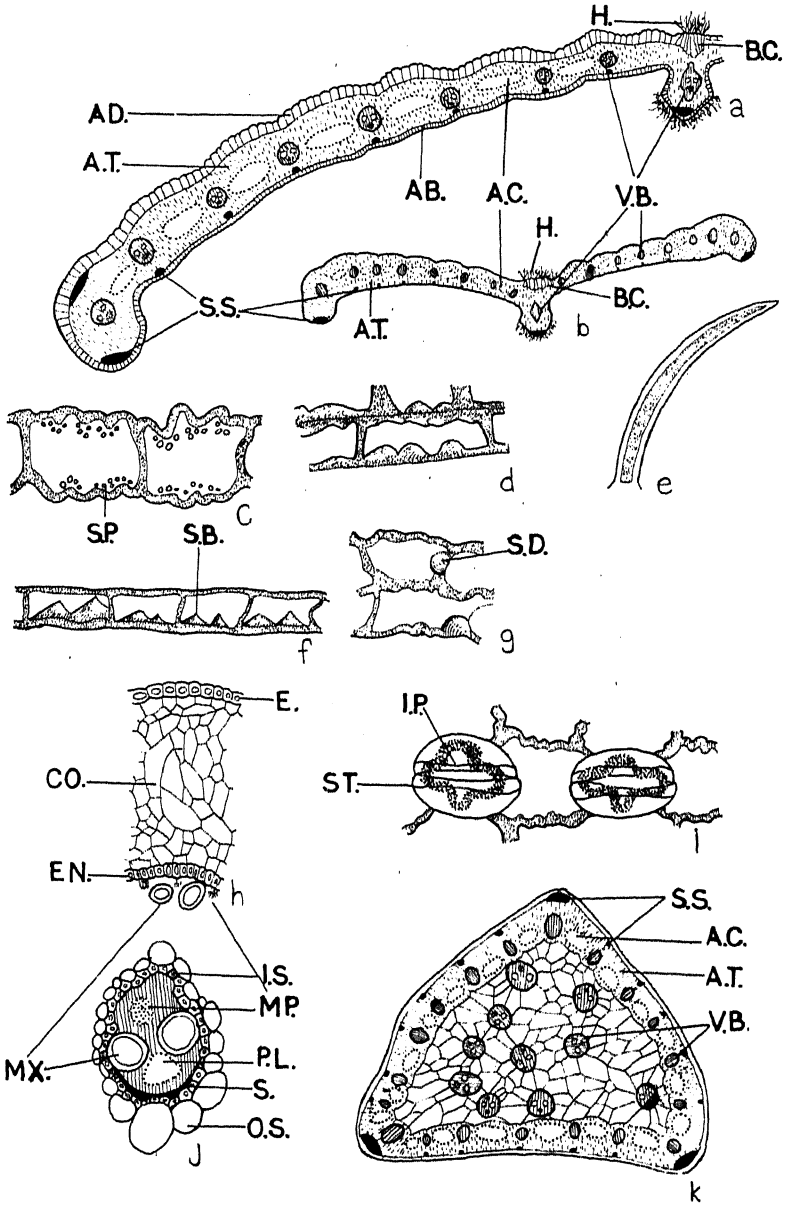
T. S. sheath: Abaxial and adaxial epidermal cells more or less similar, slightly tangentially elongated, thin-walled. Cuticle thicker on the abaxial than on the adaxial epidermis. Shallowly many ribbed on the abaxial surface. Keel distinct, somewhat triangular, rounded (figure 8b). Air-cavities *ca* 11, regularly alternating with vb's but tending to disappear towards margin. Sclerenchyma strands variable: rounded (height and width 27–31·5 μm) opposite to vb's on abaxial side (figure 8b); crescentiform (height 22·5 μm; width 45 μm); pentangular (height 13·5–31·5 μm; width 18–40·5 μm) opposite to vb's on adaxial side but existing only as strands towards margin (figure 8c). Vb's *ca* 14, large and small not regularly alternating with each other. For other details, see lamina.

Fuirena swamyii

Leaf—*Abaxial surface*: Intercostal cells moderately elongated, more or less hexagonal; cell walls thin, conspicuously sinuous. Stomata (length 43·5–52·2 μm; width 29–31·9 μm), broadly elliptic with inner pore astroporous (figure 9i); subsidiary cells low dome-shaped; interstomatal cells short with concave ends. Silica-cells short, broad, each cell containing (1–) 2 (–3) silica-bodies without satellites (figure 9f) and occurring in 1–2 more or less continuous rows; sometimes silica-bodies showing oblique arrangement; hemispherical silica deposits also present at the corners of interstomatal cells adjoining stomata (figure 9g).

Adaxial surface: Cells short, hexagonal. Silica-cells as in abaxial surface. Silica deposits and stomata absent. For other details, see abaxial surface.

T. S. lamina: Outline flanged V-shaped, symmetrical with adaxial short ribs and broad shallow median groove and thickening towards margin; keel prominent, rounded, urn shaped; margins rounded, sloping downwards (figures 9a,b). Specimen examined *ca* 4·6 mm wide. Cuticle very thin. Adaxial epidermal cells distinctly larger than those of the abaxial, the former rather thin-walled and the latter thick-walled. Hairs unicellular, unbranched, thick-walled, acute (length 69·6–116 μm; figure 9e) present on the keel and adaxial median groove. Bulliform cells radially elongated, inflated more or less translucent occurring in median grooves in groups of 7–8 cells wide (figures 9a,b). Sclerenchyma: abaxial, keel, marginal and adaxial submarginal strands (height 31·5–45 μm; width 45–90 μm), pulviniform (figures 9a,b); abaxial strands opposite to each vb (height 18–27 μm; width 27–35 μm) more or less rounded (figures 9a,b).



Figures 9a–k. *Fuirena swamyii* Govind. **a.** transection of one half of lamina (× 42); **b.** sectional view of entire lamina, diagrammatic; **c.** culm epidermal cells with silica-bodies (× 380); **d.** epidermal cells of culm (× 380); **e.** leaf epidermal hair (× 272); **f.** silica-cells in sectional view (× 380); **g.** abaxial epidermal cell with silica deposit (× 380); **h.** transection of portion of root (× 56); **i.** surface view of stomata (× 380); **j.** peripheral culm vascular bundle (× 133); **k.** transection of culm (× 28) (from Govindarajalu, 12252, *type*).

Mesophyll made up of 2 layers of palisade cells subjacent to each epidermis, the remainder usually polygonal. Air-cavities *ca* 14 in number, regularly alternating with vb's each occupied by hexagonal, thin-walled, translucent parenchyma cells. Vb's *ca* 17

in number for total width of lamina, major and minor vb's regularly alternating with each other both belonging to type III B and containing protoxylem lacunae; metaxylem vessel elements (*ca* 17 μm in diameter). Metaphloem consisting of 5–6 large sieve tube elements and fewer companion cells. Bundle sheaths double, both complete; os parenchymatous; is fibrous. Secretory cells rather common.

Culm—*Epidermis, surface view*: Cells short (slightly elongated), thick-walled, conspicuously sinuous (figure 9d). Silica-bodies wedge-shaped occurring in the sinuosities of all cells: silica-cells short, broad, isodiametric, thin-walled each containing usually 1 (2–3) silica-bodies without satellites occurring in 2–3 discontinuous rows; silica deposits of hemispherical shapes and in groups of minute bodies present on either side of anticlinal walls of cells overlying strands (figure 9c).

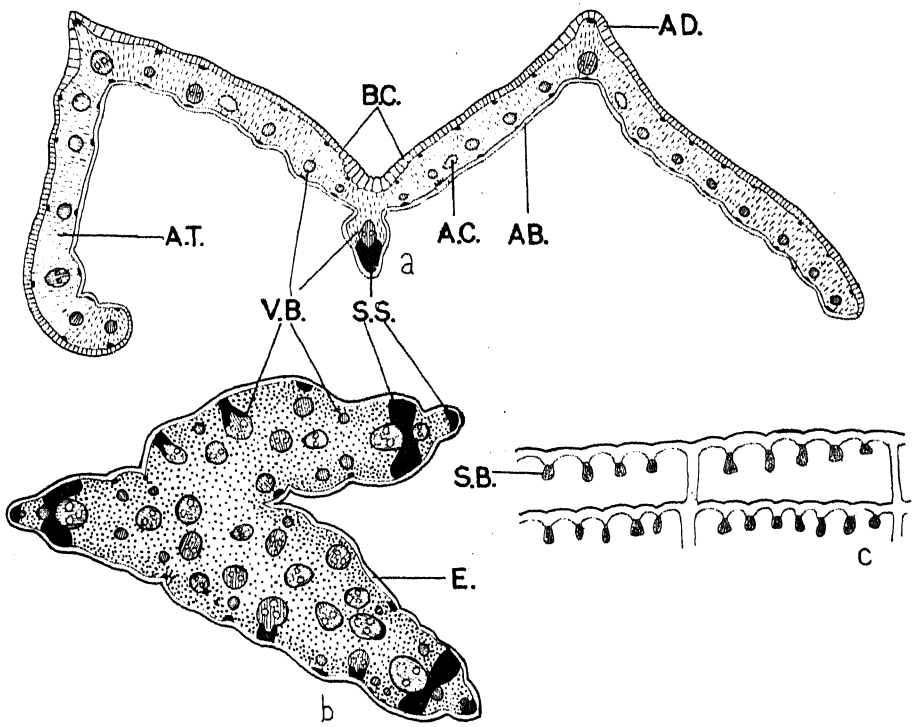
T. S. Culm: Outline triangular with slightly broad rounded corners (figure 9k). Sides in specimen examined *ca* 2–2·1 mm long. Epidermis consisting of thick-walled, isodiametric or more or less rounded cells. Cuticle *ca* 11.6 μm in thickness. Central ground tissue very spongy containing irregular air-spaces. Air-cavities peripheral forming a ring and regularly alternating with vb's (figure 9k) each containing thin-walled stellate parenchyma with elongated arms. Assimilatory tissue: chlorenchyma not radiate, composed of 3–4 layers of rounded cells. Vb's represented by an outer circle of *ca* 19 vb's and *ca* 9 additional ones embedded in spongy ground tissue; vb's oval to circular in outline, belonging to type III B with protoxylem lacunae; metaxylem vessel elements (23·2–34·8 μm in diameter). Metaphloem of 'regular type'. Bundle sheaths: double, both complete; os parenchymatous, is fibrous (figure 9j). Sclerenchyma: each vb in outer circle with more or less rounded strands (height and width 45–54 μm); strands at the corners (height 18 μm; width 58·5–63·5 μm), pulviniform (figure 9k); *ca* 2 layers of sclerenchyma forming crescentiform caps also present at xylem poles (figure 9j). Secretory cells common in the neighbourhood of vb's.

T. S. Root: Diameter of specimen examined 0·5–0·6 mm. Exodermal cells thick-walled. Cortex very spongy consisting of irregular air-spaces in it (figure 9h). Endodermis distinct consisting of broad lumened, uniformly thickened more or less radially elongated cells (figure 9h). Pericycle single layer of thick-walled cells. Central ground tissue sclerenchymatous. Xylem represented by *ca* 9 metaxylem units alternating with as many metaphloem groups; metaxylem vessel elements (18–27 μm in diameter), oval or more or less angular. Metaphloem not distinct (Govindarajalu 1968a).

Scleria swamyii

Leaf—*Abaxial surface*: Intercostal cells axially elongated; cell walls thin, sinuous. Stomata (length 36 μm; width 31·5–36 μm), more or less rounded; subsidiary cells triangular or dome-shaped; interstomatal cells short with concave ends; guard cells extended. Silica-cells usually elongated, thin-walled, rather broad, occurring in more or less 3 continuous rows each cell containing 2–3 or 4–6 cone shaped silica-bodies with satellites. Wedge-shaped silica-bodies present on the sinuosities of anticlinal walls (figure 10c); silica-bodies of varying size and shape sometimes present in the stomata. Marginal barbed prickles (length 22·5–31·5 μm) very common and their cell walls excessively thick-walled, lamellated with broad bases.

Adaxial surface: Cells axially elongated or shortly hexagonal. Stomata elliptic ovate occasionally present (length 31·5–36·0 μm; width 22·5–27 μm); subsidiary cells low



Figures 10a–c. *Scleria swamyii* Govind. **a.** transection of lamina, semidiagrammatic; **b.** transection of culm (× 37) **c.** surface view of leaf epidermal cells (× 380) (from Govindarajalu, 12515, *type*).

dome-shaped or parallel sided; guard cells much thickened. Wedge shaped silica-bodies absent. For other details, see abaxial surface.

T. S. lamina: Inversely W-shaped, asymmetrical (figure 10a) with small but prominent triangular keel having a rounded apex. One margin incurved and rounded, the other more or less straight. Midrib with shallow, broad furrow. Lamina examined *ca* 7 mm wide. Cuticle rather thin. Adaxial epidermal cells larger than those of the abaxial except those over sclerenchyma. Bulliform cells well developed and present as 1-layer of 6–7 cells in adaxial median groove; inflated cells also developed on either side of main adaxial ribs. Sclerenchyma: adaxial median girder crescentiform (height 46·4 μm; width 127·6 μm); adaxial and abaxial strands (height 34·8–46·4 μm; width 46·4–58·0 μm), usually pulviniform, sometimes rectangular present usually opposite to each vb. Mesophyll chlorenchymatous with a single adaxial layer of palisade cells the remainder made up of variously shaped cells. Air-cavities few, small, unevenly distributed, each containing lobed cells. Vb's *ca* 12–13 in each half of lamina aligned closer to abaxial than to adaxial surface. Median vb, those opposite to adaxial ribs and submarginal parts larger than the remainder; median adaxial rib and submarginal vb's (type III A), oval-shaped containing protoxylem lacunae and the remaining vb's (type I), circular in outline; metaxylem vessel elements (23·2 μm in diameter). Metaphloem of 'regular type'. Secretory cells common in mesophyll.

Culm—*Epidermis, surface view*: Cells axially elongated; cell walls thick, smooth. Stomata as in leaf but thick-walled. Barbed prickles (length 112·5–135 μm), common. Silica-cells over sclerenchyma strands, see leaf. Wedge-shaped silica-bodies absent.

T. S. Culm: Outline triangular with obtuse corners possessing variable number of furrows (deep and shallow) and ribs (figure 10b). Diameter of culm 2·4 mm broad along broadest region. Central region hollow. Cuticle very thin. Epidermal cells isodiametric, more or less variable in size. Air-cavities absent except small narrow substomatal chambers. Assimilatory tissue peripheral, 3–4 layers of chlorenchyma. Vb's 40–42 in number arranged more or less in two rings; outer ring consisting of 30–32 vb's variable in size and shape, small, circular (type I); medium sized vb's oval shaped (type III A); inner ring of large oval shaped vb's (type III B); medium and large vb's containing protoxylem lacunae; metaxylem vessel elements (D. 14·6–21·9 μm in diameter). Metaphloem of 'intermediate type'; vb's of inner ring characterized by 3–4 layers of sclerenchyma forming a crescentiform cap at phloem pole. Sclerenchyma present mostly as crescentiform girders made up of 3–4 layers of cells; sclerenchyma strands for submarginal large vb's at corners dumbell-shaped, massive, separating it from small marginal ones (height 225·6 μm; width 376·0 μm). Secretory cells occasional.

T. S. Root: Diameter of the root examined *ca* 0·8 mm wide. Exodermis consisting of 1–2 layers of cells; cells rather thick-walled, radially elongated. Cortex recognizable into two zones; outer broad, lacunose containing concentrically arranged air chambers being separated by radiating rows of parenchyma cells; inner cortex narrow, possessing 2–3 layers of thick-walled compactly arranged cells. Endodermis very distinct, cells of which uniformly thickened all around with lumen. Pericycle with a single layer of narrow thick-walled cells. Metaxylem large, central, solitary, (63·0 μm in diameter); protoxylem units 6–7 alternating with as many metaphloem groups each one of them consisting of 1–2 large sieve tube elements and 3–4 companion cells. Central ground tissue made up of thick-walled parenchyma (Govindarajalu 1975).

Dedication

Late Prof. Dr B G L Swamy who was my mentor, philosopher and guide for over two decades continues to be a source of inspiration. His versatile scholarship and varied interests and areas of specialization earned for him an eternal name in the botanical world. To such a luminary this paper is respectfully dedicated.

References

Cheadle V I and Uhl N W 1948a Types of vascular bundles in the monocotyledonae and their relations to the late metaxylem conducting elements; *Am. J. Bot.* **35** 486–496

Cheadle V I and Uhl N W 1948b The relation of metaphloem to the types of vascular bundles in the monocotyledons; *Am. J. Bot.* **35** 578–583

Govindarajalu E 1966 Systematic anatomy of South Indian Cyperaceae: *Bulbostylis* Kunth; *J. Linn. Soc. (Bot.)* **59** 289–304

Govindarajalu E 1968a Systematic anatomy of South Indian Cyperaceae: *Fuirena* Rottb.; *J. Linn. Soc. (Bot.)* **62** 27–40

Govindarajalu E 1968b Systematic anatomy of South Indian Cyperaceae: *Cyperus* L. subgen. *Kyllinga* (Rottb.) Suringar; *J. Linn. Soc.* **62** 41–58

Govindarajalu E 1975 Systematic anatomy of South Indian Cyperaceae: *Eleocharis* R. Br., *Rhynchospora* Vahl, and *Scleria* Berg.; *Adansonia* **14** 581–632

Metcalfe C R 1971 *Anatomy of the monocotyledons V. Cyperaceae*: (Oxford: Clarendon Press) pp. 597
Metcalfe C R and Gregory M 1964 Some new descriptive terms for Cyperaceae with a discussion of variations in leaf form noted in the family; *Notes Jodrell Lab.* **1** 1–11

Key to figure letterings and abbreviations used in text

AC – air-cavity
AD – adaxial epidermis
AT – assimilatory tissue
BC – bulliform cell
BS – bundle sheath
CH – chlorenchyma
CO – cortex
CU – cuticle
D – diaphragm; diaphragm cells
E – epidermis
EN – endodermis
EX – exodermis
H – hair
IC – inner cortex
IP – inner pore
IS – inner sheath
MP – metaphloem
MS – middle sheath
MX – metaxylem
OS – outer sheath
P – pericycle
PH – metaphloem
PL – protoxylem lacuna
RP – radiating parenchyma
S – crescentiform sclerenchyma
SB – silica-body
SC – secretory cell
SD – silica deposit
SP – silica particle
SS – sclerenchyma strand
ST – stoma
STC – substomatal chamber
Vb – vascular bundle (plural vb's)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 33–40.
© Printed in India.

Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L)

SUSAN EAPEN and P S RAO

Plant Biotechnology Section, Bio-Organic Division, Bhabha Atomic Research Centre, Trombay, Bombay 400 085, India

MS received 30 April 1984; revised 1 October 1984

Abstract. Callus cultures were initiated from immature embryos and seeds of breadwheat. The frequency and intensity of callus development were found to be best when embryos were oriented with the scutellum facing up and the embryonal axis in contact with the medium. Callusing frequency was also enhanced when embryos were cut longitudinally into two halves and cultured. Agar-solidified medium produced a better frequency of embryo response compared to stationary liquid cultures. Increasing levels of glucose and sucrose caused a decrease in fresh weight and a sharp increase in dry weight. Addition of coconut water, yeast extract, malt extract, amino acids and triacontanol was either without effect or were inhibitory for growth of callus. Histology of regeneration from embryo-derived callus showed *de novo* differentiation of shoot buds and roots. No evidence for somatic embryogenesis was observed.

Keywords. Callus cultures; plant regeneration; breadwheat; *Triticum aestivum*.

1. Introduction

Tissue cultures of wheat capable of differentiation have been reported by many workers (Ahloowalia 1982; Ozias-Akins and Vasil 1982; Sears and Deckard 1982; Maddock *et al* 1983). However, little attention has been focussed on the factors controlling callus induction, growth and differentiation.

In an earlier communication, callus induction and plant regeneration in tissue cultures of breadwheat were briefly reported (Eapen and Rao 1982). This communication embodies the results of detailed investigations on factors regulating callus growth and differentiation in breadwheat, *Triticum aestivum* L.

2. Materials and methods

Wheat (*Triticum aestivum* L. Thell Cvs N1–4 and Kalyan Sona) was used as the source material. Callus cultures were initiated from immature embryos and mature seeds. For culture of immature embryos, developing caryopses (14–21 days after anthesis) were collected from plants grown in fields during January–February, and surface sterilised with 70% alcohol for 30 sec, followed by 0·1 % mercuric chloride for 4 min. The seeds were rinsed five times in sterile distilled water. Embryos were dissected under a stereo microscope in a laminar flow chamber and cultured. For callus initiation from seeds, surface sterilised seeds were imbibed in sterile water for 30 min before utilising for culture.

The basal medium comprised of macro- and micro-elements of Murashige and Skoog's (1962) medium and vitamins of Lin and Staba's (1961) medium. It was supplemented with 2,4,5-trichlorophenoxy propionic acid (2,4,5-Cl_3POP) or 2,4,5-

trichlorophenoxy acetic acid (2,4,5-T) or 2,4-dichlorophenoxyacetic acid (2,4-D) (NBC) at 1,2 or 5 mg/l. The embryos were cultured either on agar-solidified (Sisco agar—0·7%) or stationary liquid medium in 5 cm petridishes each containing 4 ml of the medium. An average of 9 embryos were cultured per dish and replicated 5 times. Observations were taken at the end of 3 weeks. Cultures were incubated at $25 \pm 2°C$ under continuous light (950 lux).

For studying the effect of different treatments on callus growth, fresh and dry weight increases were taken as parameters. About 100–500 mg of tissue from first passage was transferred to the test medium and increase in fresh and dry weight was calculated after a growth period of 40 days. Initial dry weights were computed, while the final dry weight was taken after drying the tissues at 65°C for 24 hr.

Sucrose was generally used at 2% except in experiments on the effect of different carbon sources, where glucose and sucrose were tried at 2,4,6,8 and 10%. The MS medium containing 10 mg/l of 2,4,5-Cl_3POP was variously supplemented with yeast extract (YE), malt extract (ME), lysine, asparagine, glutamine and serine (Sigma Chemical Co.) to study the effect on callus growth. The effect of triacontanol, (0·1, 1 and 10 mg/l) was also investigated. For differentiation, calli were transferred to media supplemented with auxins like indoleacetic acid (IAA) or α-naphthaleneacetic acid (NAA) (0·1, 0·2, 0·5 and 1 mg/l) in factorial combinations with various cytokinins like zeatin (z), (Sigma Chemical Co.), benzyladenine (BA), 6-γ-γ-dimethylallylamino purine (2,iP) or kinetin (Kn), (NBC) at a concentration of 1 and 5 mg/l.

For histological studies, embryos after different periods of culture and callus cultures showing regeneration were fixed in FAA and processed in alcohol-xylol series. Sections were cut at 10 μm and stained with safranin-fast green.

3. Observations and discussion

3.1 *Effect of orientation of embryos*

The effect of orientation of embryos on callus initiation was studied. For this purpose embryos were placed on agar-solidified medium in three different ways.

(i) Plumule-radicle axis in contact with the medium, and scutellum facing up.
(ii) Plumule-radicle axis facing upwards and scutellum in contact with the medium.
(iii) One side of the scutellum and embryonal axis in contact with the medium.

Both the frequency and the intensity of callus development were found to be best when embryos were placed in orientation (i) and the least in orientation (iii) (table 1). Green and Phillips (1975) have reported similar results in maize embryo cultures. In Oats (Cummings *et al* 1976), for very young embryos, callus development occurred from the entire surface and an optimal orientation was not observed. In contrast, older embryos of oats showed better callusing when the scutellum was in contact with the medium than in the reverse type of orientation.

3.2 *Effect of surgical excision of embryos*

To study the effect of surgical excision on callusing, embryos were excised in two different ways.

(i) embryos were cut longitudinally into two halves in such a way that each half

Table 1. Callusing response of immature embryos of *T. aestivum* on MS+ 2,4,5-Cl_3POP (5 mg/l, growth period: 3 weeks)*.

Type of explant and its orientation	Percentage of callusing	Intensity of callusing
Scutellum facing up	95	+ + +
Scutellum in contact with the medium	90	+ +
One side of the embryo touching the medium	83	+ +
Embryo cut longitudinally into two	100	+ + +
Scutellum	33	+ +
Embryo devoid of scutellum	47	+ +

* Average of 30 embryos. + +, good, + + +, excellent.

contained the scutellum and embryonal axis. (ii) the embryonal axis and scutellum were cultured separately.

Longitudinal halves showed excellent callussing in cent per cent of cultures (table 1). However, excised scutellum and embryonal axis performed poorly as compared with the intact embryo. Freeling *et al* (1976) have noted that slicing or chopping of embryos of maize which otherwise generate totipotent cultures, resulted in apparent loss of totipotency. Tilton (1981) reported that in *Z. mays*, when the scutellum was excised and cultured, it did not develop into callus with sustained growth. In the present investigation, excision of embryos longitudinally into two halves enhanced the frequency and intensity of callus development.

3.3 *Liquid vs semi solid media*

Embryos cultured on solid medium produced a higher frequency of callusing response compared with stationary liquid culture. 2,4,5-Cl_3POP at 5 mg/l and 2,4-D at 1,2 and 5 mg/l induced cent per cent callusing on solid medium. 2,4,5-T was slightly less effective than 2,4-D or 2,4,5-Cl_3POP in the induction of callus proliferation from wheat embryos.

The growth curve of callus cultures of cv-Kalyan sona was of the sigmoid pattern (figure 1). The initial lag period occurred during the first three days of incubation followed by exponential phase which extended up to 25 days. After 25 days of culture, growth was stationary and no further increase in fresh and dry weight was recorded. Hendre *et al* (1975) have reported that in maize, wheat, rice and sorghum cultures, the initial lag period lasted for about 10–15 days and the stationary phase commenced at the end of 30–40 days. Compared with this, callus cultures of breadwheat (present study) had a faster growth which probably may be due to the difference in genotype and media formulations used in the two studies.

3.4 *Effect of different carbon sources*

For fresh weight increase glucose and sucrose proved best at 2% level. Between the two

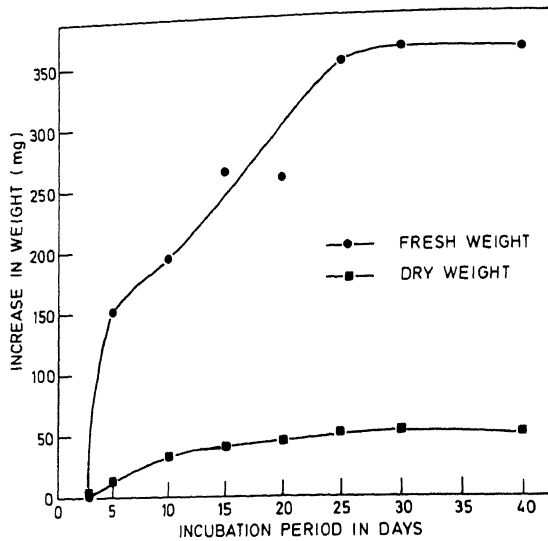


Figure 1. Growth curve based on fresh and dry weights of callus cultures of *T. aestivum* cv. Kalyan Sona grown on MS + 2,4,5-Cl_3POP (5 mg/l) at the end of 40 days of incubation.

carbohydrate sources, glucose was better than sucrose. On dry weight basis the growth of the callus was better at higher concentrations of both types of sugars, sucrose being optimum at 4% and glucose at 8 and 10% (figure 2). The increase in dry weight with increasing concentrations of sucrose may be due to deposition of heavy cell wall material. High sucrose concentration is known to favour the deposition of callose in sieve plates of callus cultures of *Glycine*, *Daucus* and *Syringa* (Aloni 1980).

3.5 *Effect of* YE *and* ME

Addition of YE or ME at 250, 500 and 1000 mg/l to MS + 2,4,5-Cl_3POP (10 mg/l) did not produce any significant alteration in fresh and dry weights compared with the control. In maize addition of 800 mg/l of YE stimulated callus growth by 40% while ME at 100–1600 mg/l was without any effect (Green 1978). Bhojwani *et al* (1977) have shown that YE inhibited callus growth of wheat.

3.6 *Effect of amino acids*

Experiments were conducted to assess the effect of various amino acids such as lysine, asparagine, glutamine and serine at 1 m M. Growth response of tissues on asparagine and glutamine containing media were comparable to those of the control whereas lysine and serine inhibited callus growth. These findings contrasts with that of maize where L-glutamine, L-serine, L-asparagine and L-lysine stimulated callus growth by 68,51,37 and 30% respectively (Green 1978).

3.7 *Effect of triacontanol*

Triacontanol at 0·1, 1 and 10 mg/l added to MS + 2,4,5-Cl_3POP (10 mg/l) did not induce any significant alteration in growth as compared to the control. This is in

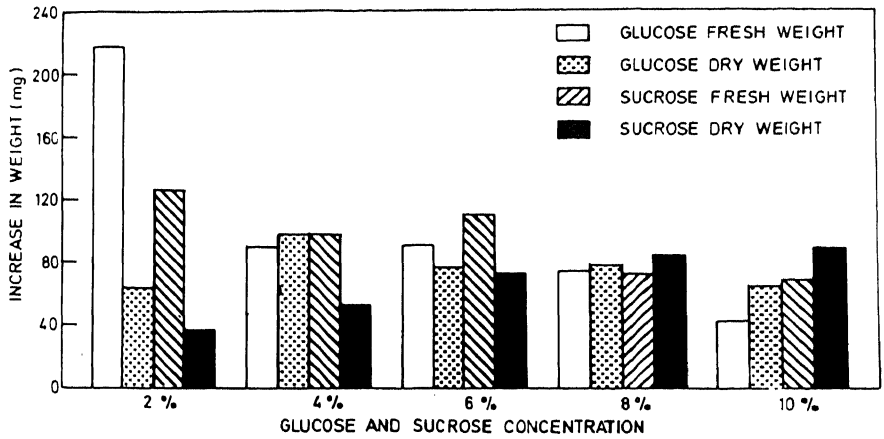


Figure 2. Effect of different concentrations of glucose and sucrose on fresh and dry weights of callus cultures of *T. aestivum* cv. Kalyan Sona grown on MS + 2,4,5-Cl_3POP (5 mg/l) at the end of 40 days of incubation.

contrast with the previous reports where triacontanol was found to be promotory for growth of plants (Ries and Wert 1977) and callus cultures of tobacco, barley and bean (Hangarter *et al* 1978). However, in breadwheat (Bole and Dubetz 1978) and durum wheat plants (Charlton *et al* 1980) triacontanol had no effect.

3.8 *Differentiation of shoot buds*

Seed callus on transfer to differentiation media produced localized green areas, which later developed into roots. The average number of roots ranged from 50 to 100 per culture. Occasionally 3–5 shoot buds were produced. However, examination of these shoot buds showed that these probably arose from pre-existing embryonal axillary shoot apices and were not induced *de novo*.

Callus cultures derived from immature embryos of N1–4 produced profuse shoot buds on transfer to basal media devoid of growth regulators. Supplementation of growth regulators to the basal medium did not enhance the frequency of differentiation. For cv-Kalyan Sona, z in combination with IAA was found to be essential for regeneration from immature embryo callus.

3.9 *Histology of shoot bud differentiation*

Histological examination of immature embryos at the time of culture showed a well developed embryonic axis with plumule, radicle, mesocotyl and scutellum (figure 3A). The scutellum had a uniformly thick epidermal layer. Beneath the epidermis, the scutellum was composed of an array of irregularly shaped parenchymatous cells. In the embryonic axis cells were smaller in size. The cells of the apical meristem were thin-walled, cytoplasmically dense and displayed a large nucleus relative to the cell size. Similar features were observed in the radicle also. Callusing was initiated from the region of coleorhiza and scutellar node (figures 3 B,C). The proliferation consisted primarily of undifferentiated parenchyma cells. In 7-day-old cultures, a callus mass

Figure 3. Histology of callus initiation and shoot regeneration in cultured embryos of *T. aestivum*. **A.** An embryo at the time of culture showing scutellum, coleoptile and coleorhiza (Sc, scutellum, Cl, coleoptile, Cr, coleorhiza). **B.** Callus initiation from the region of radicle in cultured embryo. **C.** An embryo after 4 days of culture showing callus initiation from scutellar node. **D.** Organization of root from callus. **E.** Shoot buds with apical dome flanked by leaves arising from the surface of embryo callus. Also seen below are the tracheary elements.

consisting of parenchymatous cells was prominent. There was no evidence for elongation of plumule or radicle in the majority of cases.

Histological examination of 4 week old callus cultures showed *de novo* development of shoot buds scattered all over the surface of the calli. Meristematic areas were seen to originate on the surface of the calli. The cells within these areas appeared very similar to those found in the shoot apex of the embryonal axis. Mitotic activity was more apparent within these regions relative to the surrounding areas. The original shoot buds and lateral buds remained intact and were not involved in the initiation of fresh shoot buds. The fresh shoot buds initiated were further away from the coleoptile and originated mostly towards the scutellar side. There was a clear evidence that these were produced *de novo* and not from pre-existing buds. The shoot buds originated as protuberances on the tissue surfaces and were flanked by leaves (figure 3E). Sometimes

regeneration of leaves unaccompanied by shoot buds was also observed. After further growth, vascular tissues developed below the shoot apices. Root meristems were mostly initiated deep inside the callus which later extended outwards through the callus (figure 3D). The origin of root was independent of shoot bud regeneration. No evidence for somatic embryogenesis has been observed in any of the cultures so far. In the present report, regeneration took place by organization of shoot meristems which is in agreement with previous reports in cereals (Rangan 1974; Shimada and Yamada 1979; Springer *et al* 1979).

Recently, however, somatic embryogenesis has been documented in many species of Gramineae. Typical embryoids are commonly found in *Pennisetum americanum* (Vasil and Vasil 1982), *Panicum maximum* (Lu and Vasil 1982) and *Zea mays* (Lu *et al* 1982). However, the formation of embryoids is not as evident in wheat as in other cereals. Ozias-Akins and Vasil (1982) proposed that the predominant pathway to plant regeneration in wheat was through the formation of embryoids which were atypical in organization as a result of precocious germination. In a recent report, Ozias-Akins and Vasil (1983) have obtained normal somatic embryoids in callus cultures of wheat. In cereal tissue cultures, both embryogenic and non-embryogenic calli are present and embryogenic calli is only a small fraction of individual calli (Nabors *et al* 1983). The absence of or failure to detect somatic embryogenesis in the tissue cultures of wheat (present study) may be due to the different cultural conditions or media which favoured the growth and division of non-embryogenic cell type over the other.

References

Ahloowalia B S 1982 Plant regeneration from callus culture of wheat; *Crop Sci.* **22** 405–410

Aloni R 1980 Role of auxin and sucrose on differentiation of sieve and tracheary elements in plant tissue cultures; *Planta* **150** 255–260

Bhojwani S S, Evans P K and Cocking E C 1977 Protoplast technology in relation to crop plants. Progress and problems; *Euphytica* **26** 343–360

Bole J B and Dubetz S 1978 Effects of various soil supplements and water stress on the yield and yield components of wheat; *Can. J. Soil Sci.* **58** 321–323

Charlton J, Hunter N R, Greer N A, Fritz W, Addison B M and Woodbury W 1980 The effects of triacontano and triacontanol derivatives on germination and seedling growth of leeds durum wheat; *Can. J. Plant Sci* **60** 795–797

Cummings D P, Green C E and Stuthman D D 1976 Callus induction and plant regeneration in oats; *Crop Sci.* **16** 465–470

Eapen S and Rao P S 1982 Organogenesis and plantlet formation from callus cultures of different cultivars of breadwheat (*Triticum aestivum*); *Proc. Indian Natl. Sci. Acad.* **B48** 371–377

Freeling M, Woodman J C and Cheng D S K 1976 Development potentials of maize tissue cultures; *Maydica* **21** 97–112

Green C E and Phillips R L 1975 Plant regeneration from tissue cultures of maize; *Crop Sci.* **15** 417–421

Green C E 1978 Cell and tissue cultures of maize In *Maize breeding and genetics* (New York: John Wiley & Sons Inc.) (ed.) D B Walden p. 495–515

Hangarter R, Ries S K and Carlson P 1978 Effect of triacontanol on plant cell cultures *in vitro; Plant Physiol* **61** 855–857

Hendre R R, Mascarenhas A F, Pathak M and Jagannathan V 1975 Tissue culture of maize, wheat, rice and sorghum. Part 11 Growth and nutrition of callus cultures; *Indian J. Exp. Biol.* **13** 108–111

Lin M and Staba E J 1961 Peppermint and spearmint tissue cultures. 1 Callus formation and submerged cultures; *Lloydia* **24** 139–145

Lu C and Vasil I K 1982 Somatic embryogenesis and plant regeneration in tissue cultures of *Panicum maximum* Jacq; *Am. J. Bot.* **69** 77–81

Lu C, Vasil I K and Ozias-Akins P 1982 Somatic embryogenesis in *Zea mays* L; *Theoret. Appl. Genet.* **62** 109–112

Maddock S E, Lancaster V A, Rissott R and Franklin J 1983 Plant regeneration from cultured immature embryos and inflorescence of 25 cultivars of wheat (*Triticum aestivum*); *J. Exp. Bot.* **34** 915–926

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures; *Physiol. Plant.* **15** 473–497

Nabors M W, Heyser J W, Dykes T A and Mott K J 1983 Long duration high frequency plant regeneration from cereal tissue cultures; *Planta* **157** 385–391

Ozias-Akins P and Vasil I K 1982 Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L (Wheat), Evidence for somatic embryogenesis; *Protoplasma.* **110** 95–105

Ozias-Akins P and Vasil I K 1983 Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* (wheat); *Protoplasma* **117** 40–44

Rangan T S 1974 Morphogenic investigations on tissue cultures of *Panicum miliaceum*; *Z. Pflanzenphysiol.* **72** 456–459

Ries S K and Wert V 1977 Growth response of rice seedlings to triacontanol in light and dark; *Planta* **135** 77–82

Sears R G and Deckard E L 1982 Tissue culture variability in wheat. Callus induction and plant regeneration; *Crop Sci.* **22** 546–550

Shimada T and Yamada Y 1979 Wheat plant regeneration from embryo cell cultures; *Jpn J. Genet.* **54** 379–385

Springer W D, Green C E and Kohn K H 1979 A histological examination of tissue culture initiation from immature embryos of maize; *Protoplasma* **101** 269–284

Tilton 1981 The influence of individual embryonic tissues on the morphology and development of *Zea mays* (Poaceae) germlings; *Am. J. Bot.* **68** 980–993

Vasil V and Vasil I K 1982 Characterization of an embryogenic cell suspension culture derived from inflorescence of *Pennisetum americanum* (Pearl millet Gramineae); *Am. J. Bot.* **69** 1441–1449

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 41–44.
© Printed in India.

Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species

N V R REDDY and K R RAO

Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

MS received 27 January 1984; revised 25 November 1984

Abstract. Fluchloralin (N propyl-N (2 chloromethyl-2,6-dinitro-4-trifluromethylaniline)), a dinitro-aniline type of herbicide having an inhibitory effect on light reaction, results in accumulation of higher levels of nitrite in light than in dark. In contrast, benthiocarb (S-(4-chlorobenzyl)-N-N-diethyl thiocarbamate), a non-photosynthetic herbicide and uncouplar of oxidative phosphorylation causes higher accumulation of nitrite in dark than in light in both test species. Thus the latter is effective on nitrate metabolism than on nitrite metabolism. The effect of herbicides on *Acalypha* is more pronounced than on *Tridax*.

Keywords. Dinitroaniline; nitrite; thiocarbamate; selective herbicide.

1. Introduction

Nitrite is a secondary phytotoxic substance responsible for the injury and death of plants after herbicide treatment (Klepper 1975). Herbicides showing reduction of nitrite accumulation in dark are considered to have inhibitory effects upon nitrate reduction and those causing an accumulation of nitrite in light are considered to inhibit nitrite reduction (Finke *et al* 1977). It has been postulated earlier that herbicides, especially those which inhibit photosynthesis, act by causing nitrite accumulation within the cell (Klepper 1975). Ferrari and Varner (1971) demonstrated that DNP inhibited nitrite reductase activity in photosynthetic and non-photosynthetic plant parts. The main objective of the present study was to gain greater understanding about both the non-photosynthetic as well as the photosynthetic inhibitors on nitrate metabolism in relation to two weed species which were dominantly associated in the cultivated fields of the semiarid tracts of Rayalaseema area of Andhra Pradesh.

2. Materials and methods

Two week old seedlings of *Acalypha indica* Linn. and *Tridax procumbens* L. were transplanted to experimental plots and grown under natural photoperiod (30°C day/20°C night). The third and fourth fully expanded leaves from the apex were taken for experimental analysis.

Nitrite reductase assay (E.C 1.6.6.1) was conducted by the method of Finke *et al* (1977), by placing sliced leaf tissue (0·3 g) in a 50 ml beaker with 10 ml of infiltration medium containing 100 mM potassium phosphate buffer (pH 7·5); potassium nitrate and (100 mM) tergitol NPX (0·01 %) (non-ionic) surfactant and herbicide solutions of 10, 50, 100 and 500 ppm concentrations. Untreated controls were maintained with distilled water. Vacuum infiltration was accomplished by placing the beakers with incubation medium and the leaf sample in a vacuum dessicator, drawing a vacuum of

660 nm mercury for 30 sec rapidly releasing the vacuum. The process was repeated twice and one set of beakers were kept under illumination of 15 k lux and another set in dark for 30 min. After the incubation period the total nitrite was extracted by macerating the leaf tissue at 4°C. Nitrite was determined by adding diazocoupling reagents and measuring absorbance spectrophotometrically at 540 nm (Bausch and Lamb spectronic 20 spectrophotometer). All experiments had five replicates.

3. Results

In benthiocarb treated leaves nitrite accumulated rapidly in dark over the respective control (figures 1 and 2). Fluchloralin caused a greater accumulation of nitrite in light compared to dark (figures 1 and 2). Nitrite content in untreated leaves was very low when compared to treated ones. In untreated leaves of *Acalypha* the nitrite content in both dark and light was 1·16 and 1·08 whereas in *Tridax* the same was 0·97 and 0·88 in dark and light respectively. The degree of inhibition of nitrite reduction was a function of herbicide concentration and the period of incubation. Accumulation of nitrite increases with both increasing concentrations and time (figures 1 and 2). Both chemicals inhibited nitrite reductase activity but fluchloralin showed an inhibition of both nitrate and nitrite reductase activities (figures 1 and 2). Significant amounts of nitrite was accumulated in both the weeds when an uncoupler of photophosphorylation was used in light but in benthiocarb treated leaves nitrite was accumulated more in the dark than in light.

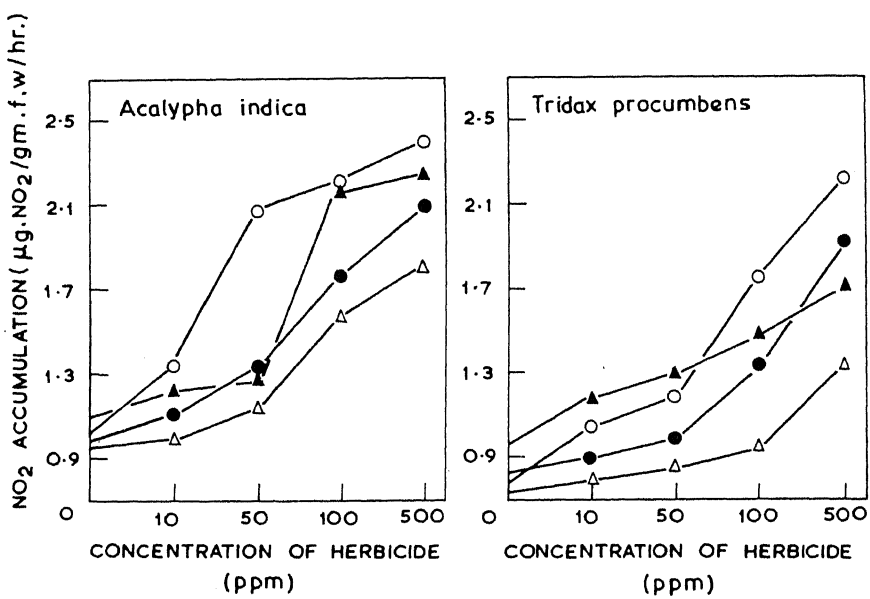


Figure 1. Nitrite recovery after extraction of weed leaf tissues with various concentrations of herbicides. Dark-(●), fluchloralin, (▲), benthocarb. Light-(o), fluchloralin, (Δ), benthiocarb.

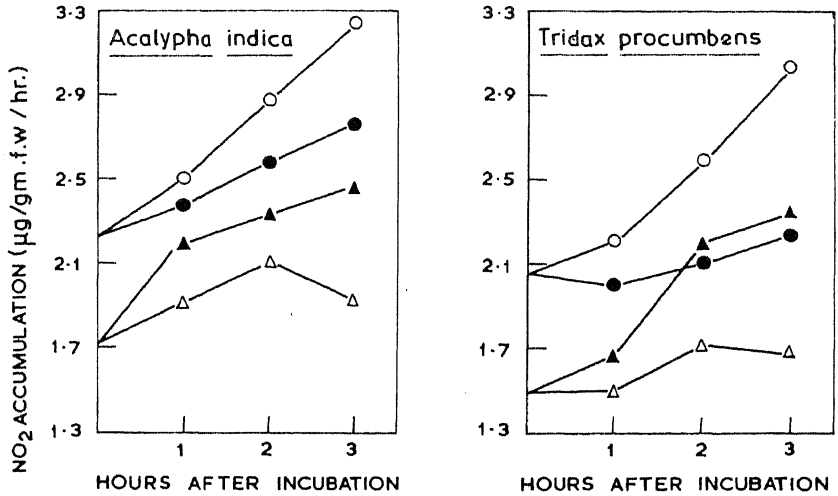


Figure 2. Accumulation of nitrite with increase in time. Dark-(●) fluchloralin, (▲)-benthiocarb. Light-(o) fluchloralin, (Δ)-benthiocarb.

4. Discussion

Since the nitrite reductase enzyme is located within the chloroplast, it is directly dependent upon reduced ferridoxin as electron donor for nitrite reduction, whereas nitrate reductase a cytoplasmic enzyme utilises a different (NADH) electron source (Klepper *et al* 1977). Ashton and Crafts (1973) reported an inhibition of nitrate reduction both in dark and light under the influence of dinoseb an uncoupler of oxidative phosphorylation which disrupts cell membranes. The increased oxidative phosphorylation will reduce the supply of NADH available for nitrate reduction (Ladonin 1966). Disruption of chloroplast membrane would result in a loss of electron flow to ferridoxin and the supply of reductant available for nitrite reduction, would decrease.

Fluchloralin, a photosynthetic inhibitor (Moreland *et al* 1972a,b) caused the accumulation of nitrite in light, possibly due to the inhibition of the supply of reductant (ferridoxin) for the reduction of nitrite. Benthiocarb, an uncoupler of oxidative phosphorylation, appeared to permit nitrate reduction in dark as the generation of NADH has been linked to the glycolytic process, which in turn would provide higher levels of NADH for nitrate reduction (Klepper 1976; Reddy *et al* 1983). This results in the formation of higher levels of nitrite within herbicide treated plants. But in light the amount of nitrite decreases due to the activity of nitrite reductase which is dependent on photosynthetically derived reduced ferridoxin.

The results presented in the text provide an evidence that certain herbicides allow nitrite to accumulate in dark. This can be explained as a continued reduction of nitrite in dark in the absence of photosynthetic nitrite reduction. But this is different in the case of fluchloralin which increases ATP consumption in dark and thus limits NADH availability for nitrate reduction. The effect of benthiocarb in dark is similar to that of photosynthetic inhibitors in light.

Acknowledgements

NVRR acknowledges the financial assistance from UGC, New Delhi. Thanks are due to Prof. V S R Das for encouragement.

References

Ashton F M and Crafts A S 1973 *Mode of action of herbicides* (New York: John Wiley) pp. 504

Ferrari T and Varner J 1971 Intact tissue assay for nitrite reductase assay in barley aleuron layers; *Plant Physiol.* **47** 709–794

Finke R L, Warner R L and Muzik J J 1977 Effect of herbicides on *in vivo* nitrate and nitrite reduction; *Weed Sci.* **25** 18–23

Klepper L A 1975 Inhibition of nitrite reduction by photosynthetic inhibitors; *Weed Sci.* **23** 188–190

Klepper L A 1976 Nitrite accumulation within herbicide treated leaves; *Weed Sci.* **24** 533–536

Klepper L A, Flesher D and Hageman R H 1977 Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves; *Plant Physiol.* **48** 580–591

Ladonin V F 1966 The effect of carbyne (Barbon) on oxidative phosphorylation of mitochondria from etiolated wild oat seedlings; *Westn. S. Kr Namki (Moscow)* **11** 137–141

Moreland D E, Farmer F S and Hussey G G 1972a Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides. I. Effect of chloroplast and mitochondrial activities; *Pest. Biochem. Physiol.* **2** 342–353

Moreland D E, Farmer F S and Hussey G G 1972b Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides. II. Effect on responses in excised plant tissues and treated seedlings; *Pest. Biochem. Physiol.* **2** 354–363

Reddy N V R, Ramaiah K R, Reddy K B and Rao K R 1983 Nitrate and nitrite reduction as influenced by S-(4-chlorobenzyl)-N,N-diethyl thiocarbamate in two tropical weed species; *Proc. Indian Acad. Sci. (Plant Sci.)* **92** 393–396

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 45–50.
© Printed in India.

Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae

T A SARMA and RAJ GARG
Department of Botany, Punjabi University, Patiala 147002, India

MS received 21 December 1983; revised 8 October 1984

Abstract. Growth and sporulation of two blue green algae *i.e.*, *Anabaena torulosa* and *Nostoc calcicola* have been studied in carbonates and bicarbonates. *A. torulosa* did not show any preference for growth in either the carbonates or the bicarbonates. In contrast, *N. calcicola* exhibited faster growth rate in carbonates and bicarbonates as evidenced by the specific growth constants and generation time. Induction of sporulation was noted by 8th day in *A. torulosa* in carbonates and bicarbonates over the basal medium (20 days). Ammonium carbonate supported the formation of maximum number of spores. However, *N. calcicola* did not show any induction of sporulation. A comparison of glycogen levels between the two algae during sporulation revealed high glycogen content with concomitant spore induction in ammonium carbonate in *A. torulosa*. Although, *N. calcicola* showed a concentration dependent increase in glycogen content in carbonates and bicarbonates, no sporulation occurred.

Keywords. Carbonates; bicarbonates; sporulation; glycogen; blue-green algae.

1. Introduction

The utilization of bicarbonate by *Anabaena* sp was reported by Keenan (1975). The incorporation of radioactive carbonate and bicarbonate by *Aphanocapsa* 6308 was demonstrated by Weathers and Allen (1978). The possibility of bicarbonate utilization and transport in the blue-green alga *Coccochloris peniocystis* was investigated by Miller and Colman (1980). The accumulation of inorganic carbon and photosynthesis were further shown to be a function of external pH in the same organism (Coleman and Colman 1981). However, there are no reports in literature on the effects of carbonates on sporulation of blue-green algae. The present paper deals with the effects of carbonates and bicarbonates on growth and sporulation in two blue-green algae.

2. Materials and methods

Clonal and axenic cultures of two heterocystous blue-green algae, *Anabaena torulosa* (our own isolate from the local rice fields; Sarma and Swarn Kanta 1979) and *Nostoc calcicola* (isolated from the 'Usar' soils of Varanasi) were grown in the nitrogen free medium of Allen and Arnon (1955). The cultures were maintained at $28 \pm 1°C$ and illuminated with day light fluorescent tubes for 14 hr/day.

Carbonates and bicarbonates of sodium and potassium and ammonium carbonate were employed with their respective chlorides as controls. The two algae were grown in various concentrations (0·1–2 mM) to determine optimum concentrations of carbon sources for growth and sporulation. Growth was measured in terms of optical density of the algal suspensions at 660 nm in a spectrocolorimeter. The initial and final pH of the cultures was noted using a digital pH meter. The pigment ratios were determined

according to Allen (1968). Samples of 10 ml cultures (with uniform optical density of 0·4) of the two algae from different nutrients were concentrated by centrifugation and the chlorophyll pigments were extracted by the addition of 10 ml acetone. The mixture was stored in a refrigerator for 4 hr after which the sample was vigorously shaken and centrifuged. The absorbancy of the chlorophyll pigments from the supernatant was measured at 660 nm. The phycobiliproteins were extracted from the residue by resuspending it in distilled water (10 ml) and subjecting it to repeated freezing and thawing. The mixture was centrifuged and the absorbancy of phycocyanins was measured at 610 nm. Specific growth constant (k) and generation time (gt) were calculated according to Kratz and Myers (1955). The number of free spores were counted in a haemocytometer.

Since the concentration of sodium or potassium varies when the respective carbonate concentration is increased, a separate set of experiments were performed where the concentration of sodium/potassium was kept constant at 2 mM, by varying the respective chloride. The glycogen content of the algal cells (expressed in μg chlorophyll), grown in the optimum concentrations of carbonates and bicarbonates, was determined at regular intervals of 4 days (Sarma and Swarn Kanta 1979). The absorbancy of chlorophyll pigments (extracted as described above) was measured at 645 and 660 nm in a spectrocolorimeter. The amount of chlorophyll was estimated by the equation given by Holm (1954).

3. Results

The optimum concentrations required for growth, growth constants and generation time of *A. torulosa* grown in carbon sources are given in table 1. Sodium chloride at a concentration of 2 mM supported maximum growth of the organism with a generation time of 13·8 hr in contrast to 17·3 hr in the control. Carbonates and bicarbonates when supplied individually did not support growth but when carbonates were supplemented

Table 1. Comparative growth data of *A. torulosa* in carbon sources.

Nutrient	pH Initial	pH Final	Optimum concentration (mM) for growth	k	gt (hr)	Pigment ratio A610/A660
Sodium chloride	7·8	7·6	2·0	0·200	13·8	1·12
Potassium chloride	7·8	7·6	2·0	0·165	16·7	1·12
Sodium carbonate	7·8	8·2	0·1	0·120	34·5	1·12
Potassium carbonate	7·8	8·1	0·1	0·122	33·9	1·20
Sodium chloride + Sodium carbonate	7·9	8·1	1·8 0·1	0·173	15·9	1·40
Potassium chloride + Potassium carbonate	7·9	8·2	1·8 0·1	0·153	17·3	1·80
Sodium bicarbonate	7·8	8·2	0·1	0·160	25·8	1·17
Potassium bicarbonate	7·9	8·2	0·1	0·089	37·0	1·20
Ammonium chloride	7·6	7·9	0·5	0·170	16·2	1·70
Control	7·6	7·9	—	0·160	17·3	1·15

Table 2. Comparative growth data of *N. calcicola* in carbon sources.

Nutrient	pH Initial	pH Final	Optimum concentration (mM) for growth	k	gt (hr)	Pigment ratio A610/A660
Sodium chloride	7·7	8·2	1	0·115	36·0	1·00
Potassium chloride	7·7	8·1	1	0·162	17·0	1·36
Sodium carbonate	9·7	8·1	2	0·164	16·8	1·44
Potassium carbonate	9·8	8·9	2	0·175	15·7	1·75
Sodium chloride + Sodium carbonate	8·0	7·6	1 0·5	0·158	17·4	1·60
Potassium chloride + Potassium carbonate	8·2	7·8	1 0·5	0·158	17·4	1·10
Sodium bicarbonate	8·7	9·0	2	0·143	19·3	1·23
Potassium bicarbonate	8·7	8·2	2	0·163	16·8	1·13
Ammonium chloride	7·6	6·6	2	0·108	38·3	4·10
Ammonium carbonate	8·2	7·5	2	0·108	38·3	2·90
Control	7·8	7·6	—	0·115	36·0	1·00

at a constant concentration of either sodium/potassium the generation time of the organism almost equalled that of the control. Thus carbonates when incorporated alone or in combination with their chlorides supported maximum growth at 0·1 mM concentration. The pH of the cultures varied with marginal increase or decrease. *N. calcicola* exhibited a concentration dependent enhancement in growth in the presence of carbonates, carbonates in combination with their chlorides and bicarbonates. Maximum growth of the organism was noted in 2 mM concentrations of carbonates and bicarbonates (table 2). The phycocyanin/chlorophyll ratios in *A. torulosa* in most of the media was ~ 1. Potassium carbonate with potassium chloride and ammonium carbonate increased the ratio to 1·7–1·8 (table 1). Likewise, *N. calcicola* also exhibited increased pigment ratios in all the carbonates. However, ammonium chloride supported maximum pigment ratio.

Although, induction of sporulation in *A. torulosa* occurred 'in all the sources of carbon' the frequency of spores in all these was almost similar or lower than the control. Ammonium carbonate at 2 mM induced maximum number of spores (table 3) within 10 days as compared to 20 days sporulation period in the control. *N. calcicola* did not show any induction of sporulation. The glycogen content of *A. torulosa* in the basal medium increased from an initial of 11·5–14 μg to a maximum of 32–37 μg. An almost similar trend persisted in case of potassium chloride with slightly higher levels of glycogen (38–42 μg). The glycogen content almost remained constant in the presence of sodium chloride. Of the two carbonates, potassium carbonate with potassium chloride supported an increase in the glycogen content upto 44·5–47 μg (figure 1A). The incorporation of ammonium chloride did not enhance glycogen content over the basal medium (30–34 μg; figure 1B). Induction of sporulation in carbonates and bicarbonates coincided with increase in the glycogen content. Ammonium carbonate supported high glycogen formation up to the 16th day. *N. calcicola* exhibited maximum accumulation of glycogen in the two carbonates (100–140 μg). However, bicarbonates did not

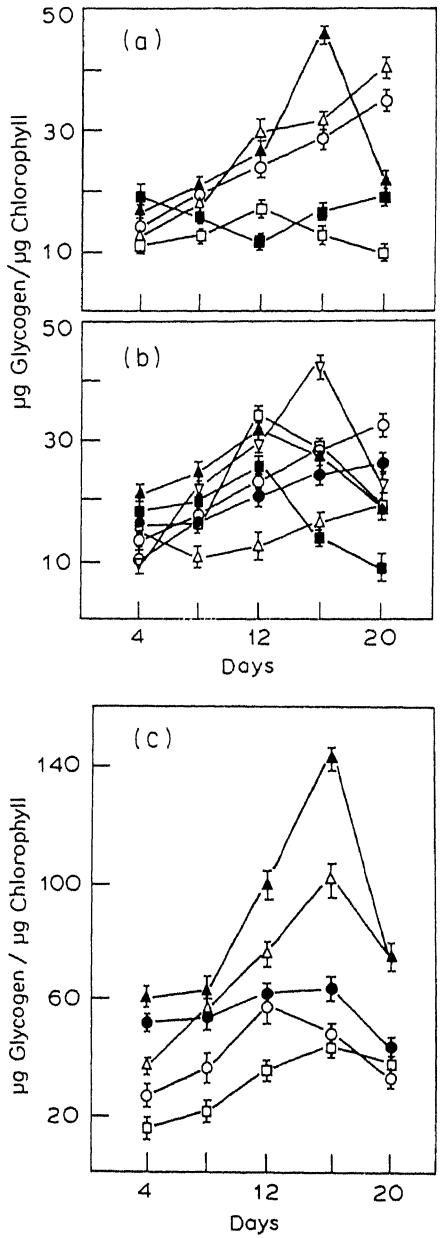
Table 3. Comparative sporulation data of *A. torulosa* in carbon sources.

Nutrient	Concentration (mM) optimum for sporulation	Sporulation period (days)	Sporulation frequency ($\times 10^4$/ml)
Sodium chloride	2	20	17·0
Potassium chloride	2	20	8·5
Sodium carbonate	2	6	2·5
Potassium carbonate	0·5	8	5·8
Sodium chloride + Sodium carbonate	1 + 0·5	—	—
Potassium chloride + Potassium carbonate	1 + 0·5	8	4·2
Sodium bicarbonate	2	10	3·7
Potassium bicarbonate	2	6	1.25
Ammonium chloride	1	6	2·4
Ammonium carbonate	2	20	2·4
Control	—	10	40·5
		20	4·5

enhance glycogen formation (figure 1C). In the rest of the media, an almost similar pattern in glycogen accumulation is noticeable where the values ranged from an initial of 9–12 μg to a maximum of 30–35 μg.

4. Discussion

Although bicarbonate utilization by *Anabaena* sp was reported by Keenan (1975), it is not known whether carbonates could also be utilized by nitrogen fixing blue-green algae. A comparative study of two blue-green algae revealed that *N. calcicola* exhibited higher growth yields in carbonates and bicarbonates, associated with higher glycogen content. On the contrary, *A. torulosa* did not respond to all the sources of carbon with respect to growth. Keenan (1975) suggested that there is no apparent relationship between photosynthesis and the presence of inorganic carbon in *Anabaena flos-aquae*. However, a correlation between pH in the range of 8 to 10 and photosynthetic activity was shown in *Coccochloris peniocystis* (Miller and Colman 1980) where most of the carbon existed as bicarbonate. The pH of the cultures of the two algae in the presence of inorganic carbon did not vary. Moreover, the phycocyanin/chlorophyll ratios indicate that the two algae photosynthesized and grew normally. The behaviour of the two algae with reference to growth, sporulation and glycogen accumulation is quite different. *A. torulosa* responded to carbonates and bicarbonates by showing induction of sporulation. Maximum number of spores were noted in ammonium carbonate. This is in agreement with the observations of Pandey and Talpasayi (1980) who showed that ammonium carbonate enhanced sporulation in *Nodularia spumigena*. Glycogen accumulation during sporulation was reported in *A. torulosa* (Sarma and Swarn Kanta 1979) and all the nutrients which favoured induction of sporulation also supported accumulation of large amounts of glycogen (Swarn Kanta and Sarma 1980; Sarma and Swarn Kanta 1980). The results presented here further demonstrate that sodium and



Figures 1a–c. **a.** Pattern of glycogen accumulation in *A. torulosa* in basal medium (○——○) and basal medium supplemented with potassium chloride (△——△), sodium chloride (□——□), sodium chloride with sodium carbonate (■——■) and potassium chloride with potassium carbonate (▲——▲). **b.** Glycogen accumulation in *A. torulosa* in basal medium incorporated with ammonium chloride (●——●), ammonium carbonate (▽——▽), sodium bicarbonate (□——□), sodium carbonate (▲——▲), potassium bicarbonate (■——■) and potassium carbonate (△——△). **c.** Pattern of glycogen accumulation in *N. calcicola* in basal medium (□——□) and basal medium supplemented with sodium bicarbonate (2·0 mM; ○——○), potassium bicarbonate (2·0 mM; ●——●), sodium carbonate (2·0 mM; ▲——▲) and potassium carbonate (2·0 mM; △——△).

potassium chlorides did not enhance the levels of glycogen but carbonates and bicarbonates enhanced glycogen content concomitant with spore induction. Ammonium carbonate which induced sporulation associated with high glycogen content in *A. torulosa* did not enhance either sporulation or glycogen content in *N. calcicola*. It would be interesting to investigate the activity of the enzymes relating to "CO_2" pump in these two algae in carbonates and bicarbonates.

Acknowledgements

The authors are thankful to the Head, Department of Botany for laboratory facilities.

References

Allen M B and Arnon D I 1955 Studies on nitrogen fixing blue-green algae. I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm.; *Plant Physiol.* **30** 366–372

Allen M M 1968 Photosynthetic membrane system in *Anacystis nidulans*; *J. Bacteriol.* **96** 836–841

Coleman J R and Colman B 1981 Inorganic carbon accumulation and photosynthesis in a blue-green alga as a function of external pH; *Plant Physiol.* **67** 917–921

Holm G 1954 Chlorophyll mutations in barley; *Acta Agric. Scand.* **4** 457–471

Keenan D J 1975 Bicarbonate utilization in *Anabaena*; *Physiol. Plant.* **34** 157–161

Kratz W A and Myers J 1955 Nutrition and growth of several blue-green algae; *Am. J. Bot.* **42** 282–287

Miller G A and Colman B 1980 Evidence for HCO_3 transport by blue-green algae (Cyanobacterium) *Coccochloris peniocystis*; *Plant Physiol.* **65** 397–402

Pandey R K and Talpasayi E R S 1980 Control of sporulation in a blue-green alga *Nodularia spumigena*; *Indian J. Bot.* **3** 128–133

Sarma T A and Swarn Kanta 1979 Biochemical studies on sporulation in blue-green algae. I. Glycogen accumulation; *Z. Allg. Mikrobiol.* **19** 569–573

Sarma T A and Swarn Kanta 1980 Biochemical studies on sporulation in blue-green algae. III. Effect of amino acids on glycogen accumulation; *Z. Allg. Mikrobiol.* **20** 647–650

Swarn Kanta and Sarma T A 1980 Biochemical studies on sporulation in blue-green algae. II. Factors affecting glycogen accumulation; *Z. Allg. Mikrobiol.* **20** 459–463

Weathers P J and Allen M M 1978 Variation in short term products of inorganic carbon fixation in exponential and stationary phase cultures of *Aphanocapsa* 6308; *Arch. Mikrobiol.* **116** 231–234

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 51–57.
© Printed in India.

Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae

V V SIVARAJAN and M C GOPINATHAN*

Department of Botany, University of Calicut, Calicut 673 635, India

* Department of Botany, University of Delhi, Delhi 110 007, India

MS received 16 September 1982; revised 8 December 1984

Abstract. The seeds of Caryophyllales are suitable materials for micromorphological studies and might possibly yield valuable taxonomic information. This paper deals with SEM studies on the seedcoat patterns of nine species of Molluginaceae. The findings support the generic delimitations and are highly useful in infrageneric classification, as well.

Keywords. Caryophyllales; Molluginaceae; seedcoat; micromorphology; systematic position; areolate; tuberculate.

1. Introduction

The circumscription of the order Caryophyllales (Centrospermae) has been a highly debated issue in the recent past. Mabry *et al* (1963) and his school emphasised pigment dichotomy in the group (Caryophyllaceae and Molluginaceae having anthocyanins and all other families with betalains) and the need for segregating them into two different orders. On the other hand Behnke (1976, 1981) and Behnke *et al* (1983) postulated that it is a homogeneous group with a common ancestry and were also supported by Nowicke and Skavarla (1977) and Mabry (1976, 1977). The nature of sieve-tube plastid elements has been largely relied upon by Dahlgren (1980), Takhtajan (1980) and Cronquist (1981) in redefining the order Caryophyllales.

Seedcoat micromorphology has provided useful information on the systematic position and interrelationships of various taxa (Brisson and Peterson 1976, 1977). Caryophyllales in general, have minute seeds with highly varied types of seed coat patterns suitable for ultrastructural studies. Despite this, little attention has been paid to this but for the work of Thieret (1966) and Wofford (1981). The results of the present investigations on some members of Molluginaceae have been encouraging and the authors propose to extend the work to other members of the group as well.

2. Materials and methods

Nine species belonging to four genera were selected for the studies. Mature seed samples selected from the herbarium specimens were coated with 100 Å of silver and were observed and photographed (S4-10 Cambridge Stereoscan). The taxa selected and the details of the voucher specimens are given below:

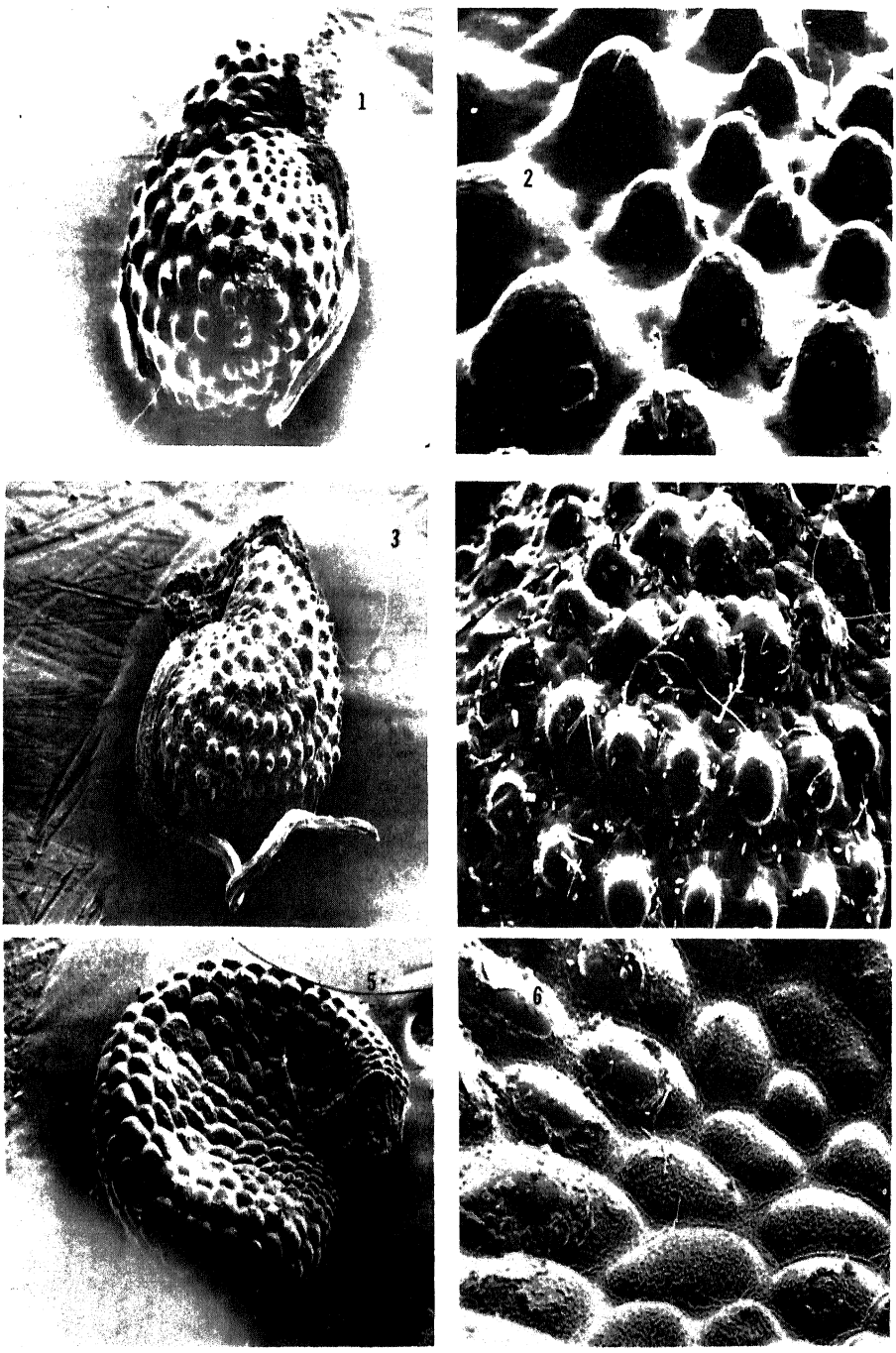
Taxa	Coll. no.	Locality deposited in	
Corbichonia decumbens (Forsk.) Exell	Chandrabose 51399	Tamilnadu	MH
Gisekia pharnaceoides L.	VVS 1300	Kerala	CALI
Glinus lotoides L.	Surendran 8691	Kerala	CALI
G. oppositifolius (L.) DC.	VVS 88	Kerala	CALI
Mollugo pentaphylla L.	VVS 428	Kerala	CALI
M. stricta L.	VVS 358	Kerala	CALI
M. disticha Ser.	VVS 887	Kerala	CALI
M. nudicaulis Lamk.	VVS 1273	Kerala	CALI
M. cerviana (L.) Ser.	VVS 891	Kerala	CALI

3. Observations and discussion

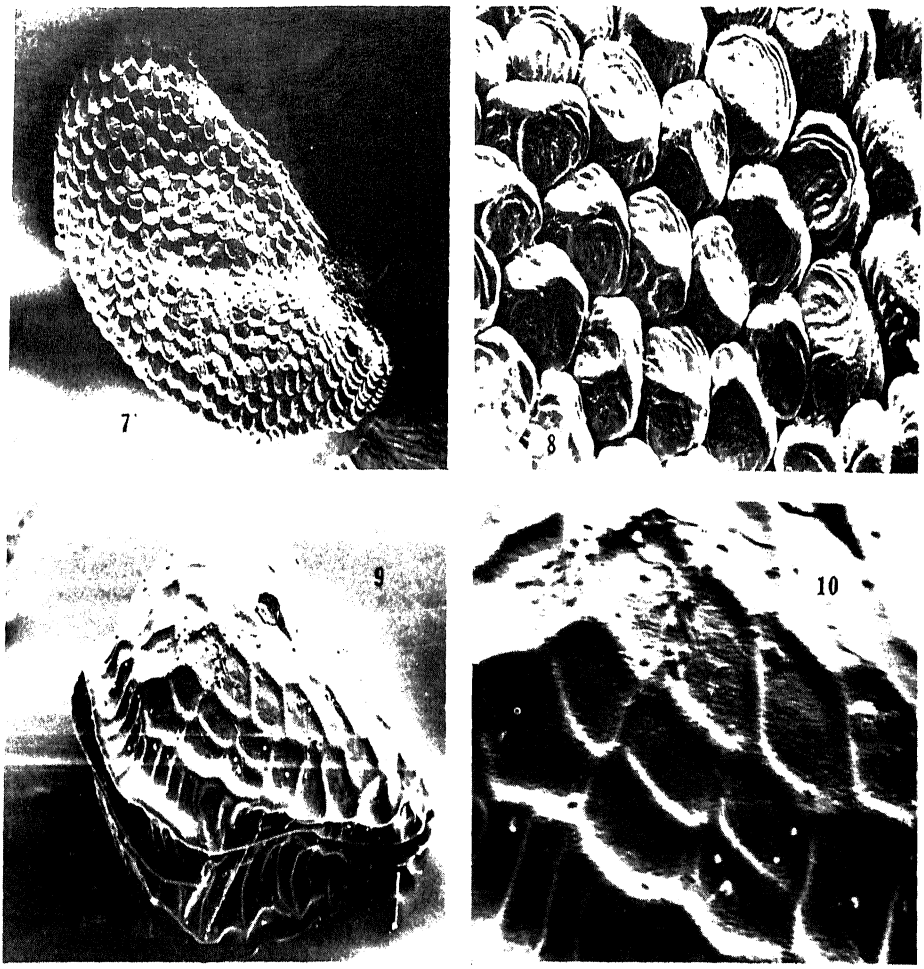
In general, the seeds of the taxa examined are minute, pale brown to brownish black and reniform or konch-shaped. Observations using SEM revealed very distinctive seedcoat patterns almost corresponding to the existing generic delimitations within the family. Thus, the genus *Glinus* had tuberculate/verrucate seeds, *Mollugo* areolate/reticulate ones, *Gisekia* punctate and *Corbichonia* costate ones. In all samples examined, the seeds had a waxy coating on their surface.

The generic status of *Glinus* L. has been a matter of discussion, until recently. It is closely similar to *Mollugo* L in its macromorphological characters and several authors treated them as congeneric (Bentham and Hooker 1867; Clarke 1879) even after Fenzl (1836) demonstrated an essential difference in their seed structure—the seeds of the former having an unequal pair of appendages called podosperms at the hilum while they are absent in the seeds of *Mollugo*. Contemporary taxonomic opinion is in favour of keeping them as two distinct genera and finds support from the seedcoat pattern studies, as well. The two Indian species of the genus studied differ among themselves in the seedcoat micromorphology. In *Glinus oppositifolius* they are tuberculate with cylindric, pillar-like tubercles in concentric circles around the hilum, the larger ones near the margins and the smaller ones towards the hilum. The waxy coating is more conspicuous in the inter-tubercular areas than on the tubercles themselves (figures 1–2). The seeds of *G. lotoides* though very similar under light microscope, appear quite distinct under SEM. They are verrucate with the ellipsoid verrucae transversely oriented to the long axis of the seeds. They are concentrically arranged around the hilum and are increasingly smaller towards it (figures 3–4). This difference in the seedcoat patterns may be of decisive importance in the circumscription and delimitation of species because overlapping variations in the macromorphological characters and intermediates have already been reported in this species pair (Backer 1951).

Of the four species of the genus *Mollugo* studied, *M. cerviana* and *M. nudicaulis* are very distinct and isolated in their general morphology and in their seedcoat patterns. *M. cerviana* has planoconvex, obconic seeds with prominently reticulate seedcoat. Meshes of the reticulum are square or polygonal with mostly oblique walls. Along the margins the meshes are deeper. The entire seed surface is studded with minute grannular excrescences (figures 9–10). In *M. nudicaulis* the seed surface is closely packed with uniformly distributed, pebble-like, lyrate and chipped areoles. Those along the



Figures 1–6. 1 and 2. *Glinus oppositifolius* (**1** × 120, **2** × 1200). **3 and 4.** *Glinus lotoides* (**3** × 120, **4** × 360). **5 and 6.** *Mollugo pentaphylla* (**5** × 60, **6** × 600).

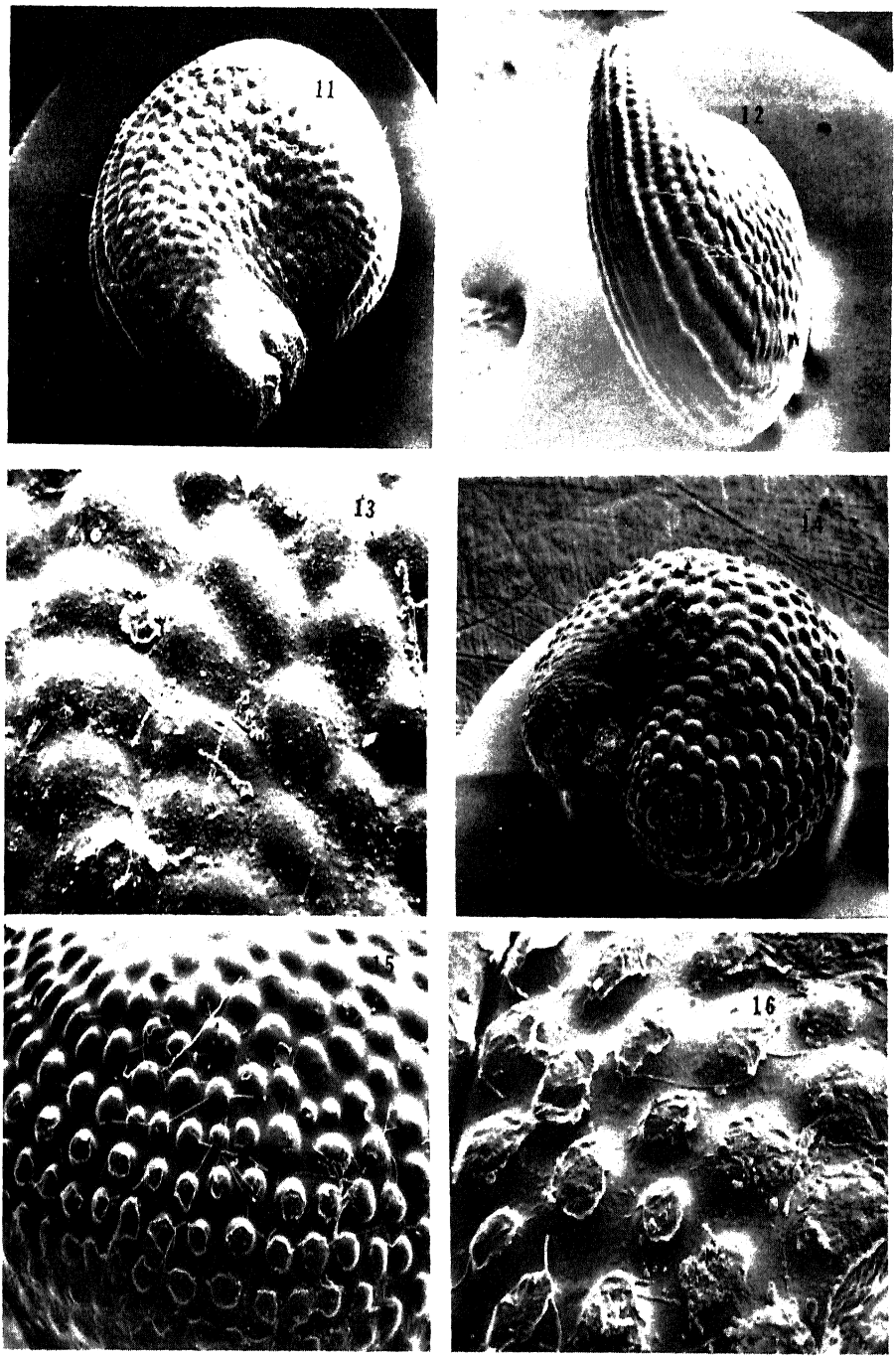


Figures 7–10. **7 and 8.** *M. nudicaulis* (7 × 120, 8 × 600). **9 and 10.** *M. cerviana* (9 × 240, 10 × 600).

margins are ellipsoid and are not chipped. The lyrations on the surface of the areoles is apparently due to differential deposition of the waxy material (figures 7–8).

M. pentaphylla has been understood as a highly variable species until recently and included in it two different elements described under two binomials by Linnaeus. Of them, *M. pentaphylla* (sensu stricto) is characterised by obovate-obtuse leaves and lax panicles and *M. stricta* by lanceolate, acute leaves with compact panicles. Observations on the seedcoat patterns have corroborated the Linnaean concept of these species. *M. pentaphylla* has areolate seeds with polygonal areoles and the entire seedsurface is covered with a microreticulum formed apparently by the deciduous waxy coating (figures 5–6). On the contrary, those of *M. stricta* are tuberculate with no such finer ornamentations as seen in the former (figures 14–16). Consequently, Sivarajan and Usha (1983) have resurrected *M. stricta* as a distinct species.

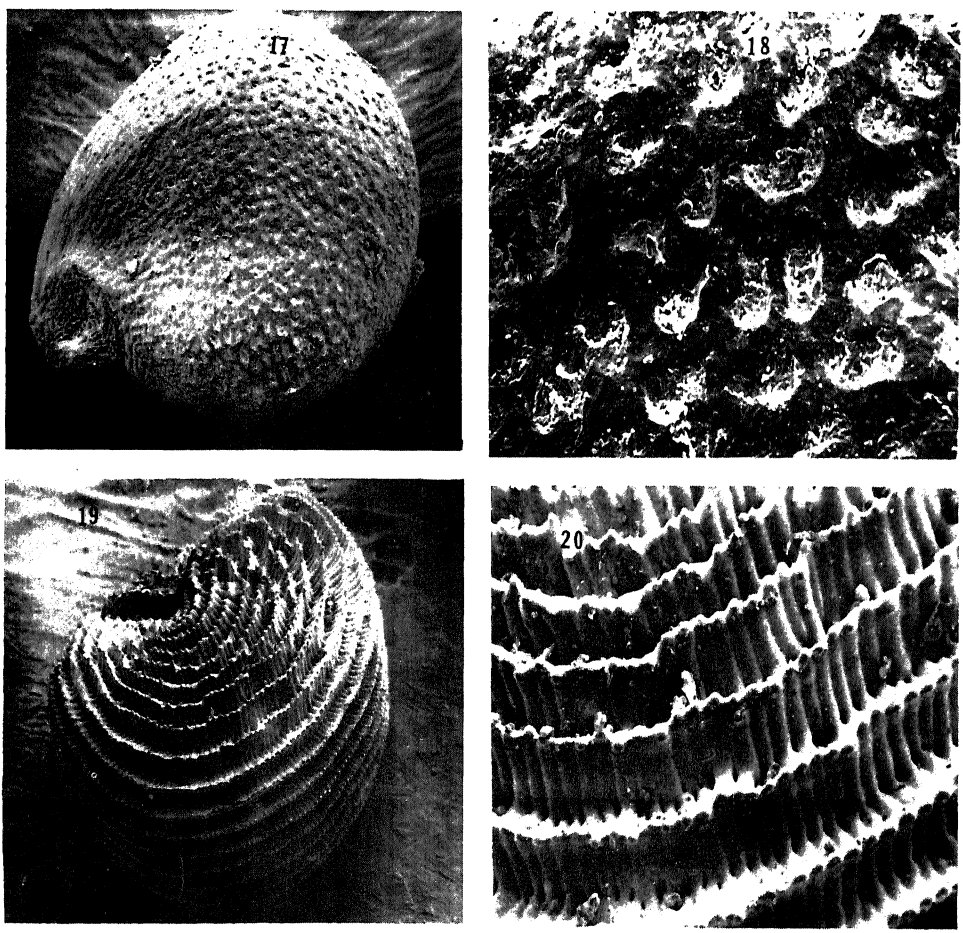
M. stricta and *M. disticha* are closely related species and are generally distinguished



Figures 11–16. **11–13.** *M. disticha* (**11** × 95, **12** × 90, **13** × 600). **14–16.** *M. stricta* (**14** × 60, **15** × 240, **16** × 600).

by their inflorescence and seed characters, the former having corymbosely branched panicles and unmargined seeds and the latter racemosely branched inflorescence and strongly margined seeds, although Clarke (1879) has reported overlapping variations in the nature of the inflorescence. However, the seeds are quite distinctive. Unlike the tubercled seeds of *M. stricta*, those of *M. disticha* are areolate and are strongly margined with prominent ridges and grooves along the margins (figures 11–13).

The systematic position of the genus *Gisekia* L. has been a matter of discussion and controversy and has been placed under different families by different authors. It is closely similar to Molluginaceae on the one hand but is unnatural here because of its 1-ovuled, apocarpous pistil and betalain pigments. These characters relate it better with Phytolaccaceae and so, Takhtajan (1980) has transferred this genus to the latter family. Present studies have revealed that the single species of this genus in India—*G. pharnaceoides*—has a punctate seedcoat (figures 17–18) and in this respect too, it has no close relative in Molluginaceae.



Figures 17–20. 17 and 18. *Gisekia pharnaceoides* (**17** × 80, **18** × 520). **19 and 20.** *Corbichonia decumbens* (**19** × 56, **20** × 220).

Corbichonia decumbens, earlier described under the genus *Glinus*, is unique in having petalloid staminodes (Clarke 1879) often confused with petals (Wight and Arnott 1834). Quite unlike those of *Glinus*, the seeds of this taxon do not have the hilar appendages and have a very different seedcoat pattern. They are prominently costate with deep intervening grooves. The costae are concentrically arranged and are narrowed from base upwards to a sharp serrate crest. They also have closely placed, regularly arranged, transverse ridges, continuous between successive crests (figures 19–20).

Acknowledgements

The authors are thankful to Mr S M Lal, Textile Technology Division, IIT, Delhi for the electron micrographs.

References

Backer 1951 *Molluginaceae*: in *Flora Malesiana* (ed.) C G G J van Steenis (Djakarta: Noordhoff) **4** 267–275

Behnke H D 1976 Ultrastructure of sieve-element plastids in Caryophyllales (Centrospermae). Evidence for the delimitation and classification of the Order; *Plant Syst. Evol.* **126** 31–54

Behnke H D 1981 Sieve-element characters; *Nord. J. Bot.* **1** 381–400

Behnke H D, Liliana Pop and Sivarajan V V 1983 Sieve-element plastids of Caryophyllales: Additional investigations with special reference to the Caryophyllaceae and Molluginaceae; *Plant Syst. Evol.* **142** 109–115

Bentham G and Hooker J D 1867 Ficoideae: in *Genera Plantarum* (London: Reeve & Co.) **1** 851–859

Brisson J D and Peterson R L 1976 A critical review of the use of SEM in the study of seedcoat; *Illinois Inst. Tech. Res. Inst.* SEM/1976/II 477–495

Brisson J D and Peterson R L 1977 The SEM and x-ray microanalysis in the study of seeds. A bibliography covering the period of 1967–1976. *Illinois Inst. Tech. Res. Inst.* SEM/1977/II 697–712

Clarke C B 1879 Ficoideae: in *Flora of British India* (ed.) J D Hooker (Kent: Reeve & Co.) II 658–664

Cronquist A 1981 *An integrated system of classification of flowering plants* (New York: Academic Press)

Dahlgren R M T 1980 A revised system of classification of Angiosperms; *Bot. J. Linn. Soc.* **80** 91–124

Fenzl E 1836 Monographie der Mollogineen und Steudelieen; *Ann. Wiener Mus. Naturgesch.* **1** 337–384

Mabry T J 1976 Pigment dichotomy and DNA-RNA hybridisation data for Centrospermous families; *Plant Syst. Evol.* **126** 79–94

Mabry T J 1977 The order Centrospermae; *Ann. Miss. Bot. Gard.* **64** 210–220

Mabry T J, Taylor A and Turner B L 1963 The betacyanins and their distribution; *Phytochemistry* **2** 61–64

Nowicke J W and Skavarla J J 1977 Pollen morphology and the relationship of the Plumbaginaceae, Polygonaceae and Primulaceae to the order Centrospermae; *Smiths. Contrib. Bot.* **37** 1–64

Sivarajan V V and Usha T 1983 On re-instating the Linnaean species *Mollugo stricta* (Molluginaceae); *Taxon* **32** 123–126

Takhtajan A 1980 Outline of the classification of flowering plants (*Magnoliophyta*); *Bot. Rev.* **46** 225–359

Thieret J W 1966 Seeds of some United States Phytolaccaceae and Aizoaceae; *Sida* **2** 352–360

Wight R and Arnott G A 1834 *Prodromus florae peninsulae Indiae Orientalis* (London: Taylor and Francis)

Wofford B E 1981 External seed morphology of *Arneria* (Caryophyllaceae) of Southeastern United States; *Syst. Bot.* **6** 126–135

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 59–64.
© Printed in India.

Nitrogen fixation by *Nostoc* strains

N JAYASANKARI and S SHANMUGASUNDARAM
Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021, India

MS received 1 August 1983; revised 12 October 1984

Abstract. Seven strains of *Nostoc* were examined for their nitrogen fixing potential in the presence and absence of NO_3^-, NH_4^+ and oxygen. The strains not only showed differences in their nitrogen fixing potential, but also with respect to their response to combined nitrogen and oxygen. Based on their nitrogen fixing potential the strains were grouped as low, moderate and high fixers.

Keywords. Cyanobacteria; acetylene reduction; ammonium and nitrate inhibition.

1. Introduction

Biological nitrogen fixation is inhibited by exogenous NO_3^- and NH_4^+ (Brill 1975; Shanmugam and Morandi 1976). NH_4^+ inhibits nitrogen fixation in *Anabaena cylindrica* when incubated in 50 μM to 5 mM (Rowell *et al* 1977; Ohmori and Hattori 1978) and in *Nostoc muscorum* in 3 mM concentration (Scherer *et al* 1980 a, b). Nitrate also inhibits the nitrogenase activity (Stewart *et al* 1968; Stewart 1980). While we do have considerable information on specific species, no attempt seems to have been made on a comparative basis within an interspecific complex. The present communication is an attempt in this direction with seven species of *Nostoc*.

2. Materials and methods

2.1 *Strains used*

Nostoc sp MKU-1*

N. carneum ARM 211 and *N. muscorum* ARM 221 were obtained from Prof. G S Venkataraman, Division of Microbiology, IARI, New Delhi, India.

N. endophilia A124, *N. ellipsosporum* A127, *N. muscorum* and *N. parameloides* A620 were obtained from the Botany department, Madras University.

2.2 *Growth*

The algal strains were grown in Allen's medium (Allen 1968) with or without combined nitrogen and suitably buffered at $25 \pm 2°C$ under 1500–2000 lux with a light dark regime of 12L : 12D.

* *Nostoc* sp MKU-1 is an isolate of MK University

2.3 *Heterocyst frequency*

The frequency of heterocysts was determined microscopically and expressed as percentage of vegetative cells. Care was taken to avoid broken filaments.

2.4 *Nitrogenase activity*

Nitrogenase activity was assayed by acetylene reduction activity. Exponentially growing cells were harvested and taken in a serum bottle of 6 ml capacity and closed with a subaseal rubber septum. Acetylene was injected to give a final concentration of 20%. Anaerobic condition when required was created by replacing the total volume of air in the serum bottle with argon. No CO_2 was added to these bottles as sodium carbonate in the medium served as the carbon source. Samples were incubated for 60 min under 1500 lux intensity. The reaction was terminated by injecting 0·2 ml of 20% TCA. 1 ml of the gas sample was analysed using 5830A Hewlett Packard microprocessor attached gas chromatograph fitted with a flame ionisation detector and a porapak column (4 feet long, 80–100 mesh).

2.5 *Ammonium and nitrate inhibition of ARA*

Various concentrations of ammonium chloride (from 1–10 mM) were used to study the influence of ammonium over acetylene reduction assay in *Nostoc* sp. Following Dharmawardene and Stewart (1973) ammonium chloride at 2 mM and sodium nitrate at 10 mM (Ohmori and Hattori 1972) were used to observe the influence on ARA in seven strains of *Nostoc*.

2.6 *Protein estimation*

Protein was estimated following Lowry *et al* (1951) after digesting the sample with 0·5 N NaOH.

3. Results

The frequency of heterocyst among the dinitrogen grown cultures of *Nostoc* strains varied between 5–9·3% (table 1). When NH_4^+-N (5 mM) grown heterocyst free

Table 1. Acetylene reduction assay and heterocyst frequency among the dinitrogen grown strains of *Nostoc*.

Strains**	Acetylene reduction* (nmol C_2H_4 mg protein^{-1} hr^{-1})	Heterocyst frequency (%)
Nostoc sp MKU-1	112·05 ± 13·67	6·9
N. carneum ARM 211	116·27 ± 15·92	5·9
N. muscorum ARM221	20·8 ± 6·54	5·71
N. endophilia A124	173·84 ± 12·06	7·68
N. ellipsosporum A127	93·38 ± 6·66	6·66
N. muscorum	32·1 ± 2·80	5·0
N. parameloides A620	186·02 ± 11·05	9·3

* Mean ± SD of the mean of three replicates, **10 day old cultures were used.

filaments were transferred to dinitrogen condition, ten day old cultures reduced the maximum acetylene in all the seven strains of *Nostoc* (figure 1) when compared to the 5 and 15 day old cultures. The heterocyst production also showed a similar pattern (table 1).

Based on the N_2 fixing capacity under oxygenic and anoxygenic conditions, the strains could be grouped as high fixers, moderate fixers and poor fixers. *N. parameloides* A620 and *N. endophilia* A124 are high nitrogen fixers, *Nostoc* sp MKU-1, *N. carneum* ARM211 and *N. ellipsosporum* A127 moderate fixers and *N. muscorum* ARM221 and *N. muscorum* poor fixers (figure 2). There was a slight stimulation of nitrogenase activity in *Nostoc* sp MKU-1, *N. carneum* ARM211 and *N. ellipsosporum*

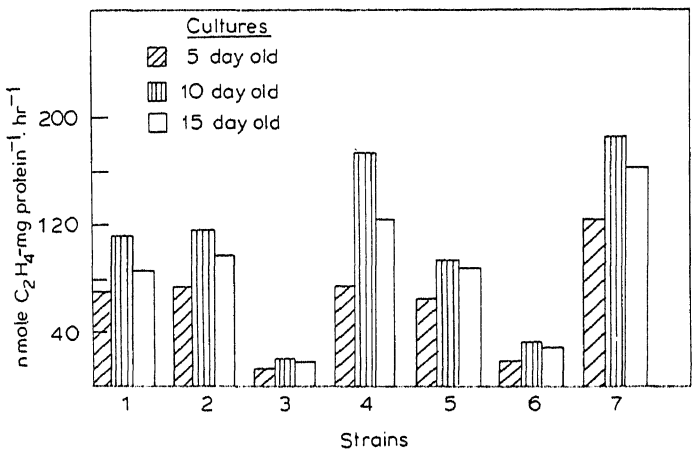


Figure 1. Acetylene reduction assay during different time intervals of growth in *Nostoc* strains.

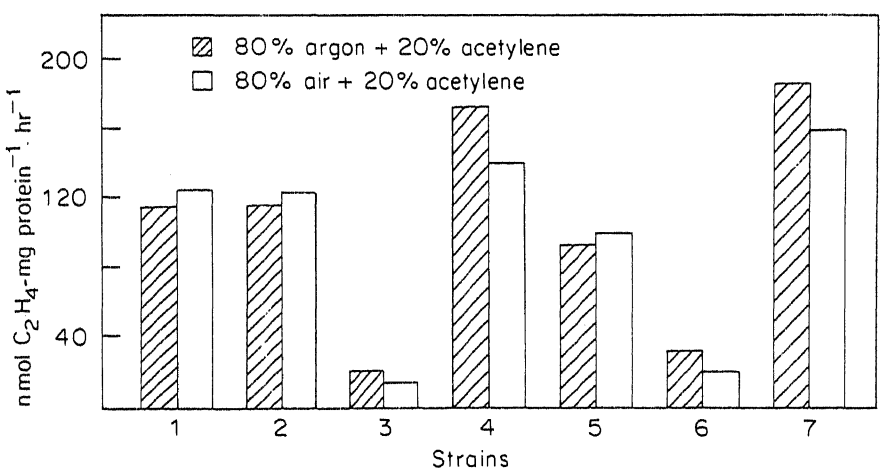


Figure 2. Acetylene reduction assay under air and argon atmosphere in *Nostoc* strains. 1. *N.* sp MKU-1, 2. *N. carneum* ARM211, 3. *N. muscorum* ARM 221, 4. *N. endophilia* A124, 5. *N. ellipsosporum* A127, 6. *N. muscorum*, 7. *N. parameloides* A620.

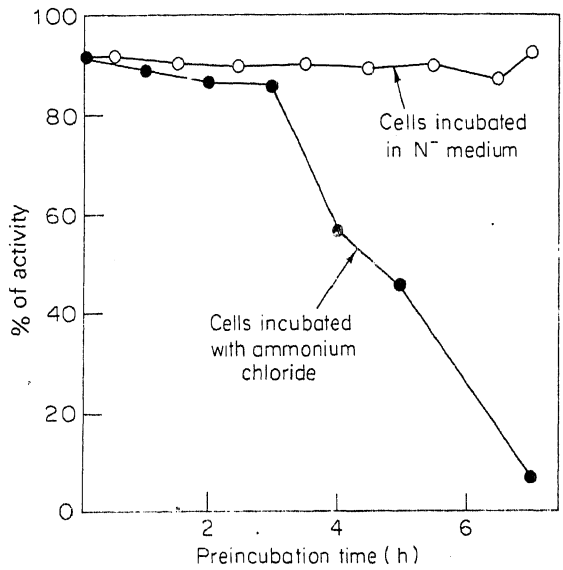


Figure 3. Kinetics of ammonium inhibition over acetylene reduction assay in *Nostoc* sp MKU-1.

A127 under aerobic condition as compared to anaerobic condition, suggesting the involvement of aerobic respiration. Figure 3 shows the effect of addition of 2 mM NH_4Cl on the nitrogenase activity in *Nostoc* sp MKU-1 as a function of time. Up to 3 hr, NH_4^+ did not have any appreciable effect, after which there was a sharp decline in the activity. At the end of 7 hr, the activity was only 7·34% that of dinitrogen grown cultures (figure 3).

Interestingly, in high fixers such as *N. parameloides* A620 and *N. endophilia* A124, addition of NH_4^+ (2 mM NH_4Cl) brought about a drastic reduction in nitrogenase activity, which was 12–13% of that in the dinitrogen growing cultures (figure 4). On the other hand, in *Nostoc* sp MKU-1, *N. carneum* ARM211 and *N. ellipsosporum* A127 (moderate fixers) and *N. muscorum* ARM221 and *N. muscorum* (low fixers), the activity was of the order of 62–76%. The strains responded differently to added NO_3-N also (figure 4). The interesting effect of NO_3^- was much more with high and low fixers than with moderate fixers.

4. Discussion

When *Nostoc* strains were compared for their nitrogen fixing capacity at different time intervals of growth, (5th, 10th and 15th day old nitrogen fixing cultures) 10 day old cultures formed maximum n moles of ethylene mg protein^{-1} hr^{-1}. Although heterocysts frequency in any strain could be a fair mensum of its nitrogen fixing potential (Stewart *et al* 1968) the condition of the heterocysts seems to be important. While the heterocyst frequency in the present *Nostoc* strains varied only by a factor of 1·5, the nitrogenase activity (acetylene reduction) varied by a factor of 5·8 (table 1). The difference in nitrogenase activity may also be due to the levels of PSII activity, levels of

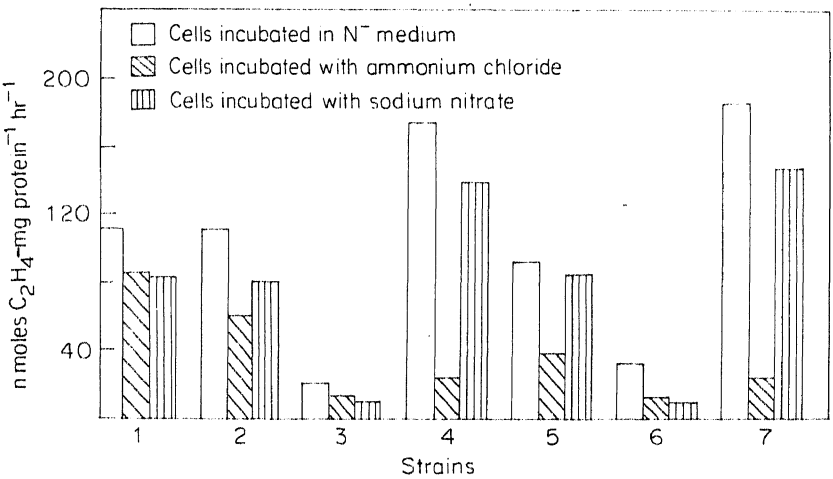


Figure 4. Acetylene reduction assay in the presence and absence of ammonium chloride and sodium nitrate in *Nostoc* strains.
1. *N.* sp MKU-1, 2. *N. carneum* ARM211, 3. *N. muscorum* ARM221, 4. *N. endophilia* A124, 5. *N. ellipsosporum* A127, 6. *N. muscorum*, 7. *N. parameloides* A620.

ammonium assimilation enzymes, levels of carbon compounds that may come from the vegetative cells to heterocyst. Low fixers and high fixers showed increase in acetylene reduction activity as in *N. muscorum* under argon (Scherer *et al* 1980). However, argon incubation reduced the acetylene reduction in moderate fixers, indicating the participation of aerobic respiration in addition to photosynthesis.

As in other cyanobacteria, (Stewart *et al* 1968; Stewart 1980) exogenous NO_3^- and NH_4^+ inhibited the nitrogenase activity, though the effect of NH_4^+ was more drastic than NO_3^-. It is interesting to note that the high fixers were more sensitive to NH_4^+ than the low fixers.

The present study shows not only a variation in the nitrogen fixing potential among different strains of *Nostoc*, but also a differential response to exogenous NO_3^- and NH_4^+.

Acknowledgement

Grateful thanks are due to CSIR, New Delhi, India for the award of a Fellowship to NJ.

References

Allen M M 1968 Simple conditions for the growth of unicellular blue-green algae on plates; *J. Phycol.* **4** 1–4

Brill W J 1975 Regulation and kinetics of bacterial nitrogen fixation; *Ann. Rev. Microbiol.* **29** 109–129

Dharmawardene M W N and Stewart W D P 1973 Nitrogen fixation by photosynthetic microorganisms; *Ann. Rev. Microbiol.* **27** 283–316

Lowry O H, Rosebrough N J, Farr A C and Randall R J 1951 Protein measurement with the folin-phenol reagent; *J. Biol. Chem.* **193** 265–275

Ohmori M and Hattori A 1972 Effect of nitrate on nitrogen fixation by the blue-green alga *Anabaena cylindrica; Plant Cell Physiol.* **13** 589–599

Ohmori M and Hattori A 1978 Transient change in the ATP pool of *Anabaena cylindrica* associated with ammonia assimilation; *Arch. Microbiol.* **117** 17–20

Rowell P, Enticott S and Stewart W D P 1977 Glutamine synthetase and nitrogenase activity in the blue-green alga *Anabaena cylindrica; New Phytol.* **79** 41–54

Scherer S, Kerfin W and Boger P 1980a Regulatory effect of hydrogen on nitrogenase activity of the blue-green alga (cyanobacterium) *Nostoc muscorum; J. Bacteriol.* **141** 1037–1040

Scherer S, Kerfin W and Boger P 1980b Increase of nitrogenase activity in the blue-green alga *Nostoc muscorum* (cyanobacterium); *J. Bacteriol.* **144** 1017–1023

Shanmugam K T and Morandi C 1976 Aminoacids as repressors of nitrogenase biosynthesis in *Klebsiella pneumoniae; Biochim. Biophys. Acta.* **437** 322–332

Stewart W D P, Filzgerald G P and Burris R H 1968 Acetylene reduction by nitrogen fixing blue-green algae; *Arch. Microbiol.* **62** 336–348

Stewart W D P 1980 Some aspects of structure and function in nitrogen fixing cyanobacteria; *Ann. Rev. Microbiol.* **34** 497–536

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, No. 1, March 1985, pp. 65–82.
© Printed in India.

Pharmacognostic studies on 'Dravanti' part-I* *Jatropha curcas* Linn.

R C GUPTA
National Botanical Research Institute, Lucknow 226001, India

MS received 6 January 1984; revised 26 December 1984

Abstract. 'Dravanti', an important Ayurvedic drug, has variously been ascribed to different botanical taxa, *Jatropha curcas* Linn. being one such species. A detailed pharmacognosy of the vegetative parts of the above species has been dealt with in the present paper. The study has revealed that such characters as the presence of 9 leaf traces in the basal parts of the petiole, mixed anisocytic and paracytic stomata with stomatal index 7·3 and 12 for the upper and the lower leaf surfaces respectively, funnel-shaped multiseriate phloem rays demarcating the phloem into wedge-shaped masses in both the root and the stem, diffused porous wood with diffused apotracheal parenchyma and 1-3 seriate xylem rays along with certain physico-chemical constants and fluorescence tests can provide useful parameters for differentiating this species from other implicated taxa.

Keywords. Dravanti; *Jatropha curcas*; pharmacognosy; Euphorbiaceae.

1. Introduction

'Dravanti' is one of the important Ayurvedic drugs, particularly known for cathartic properties (Kirtikar and Basu 1933; Narahari 1933; Bhavmisra 1949a; Charak 1949; Vagbhatt 1950; Sushrut 1952; Vaishya 1953). Botanically it has been variously referred to *Jatropha curcas* Linn. (Dutt 1922; Kirtikar and Basu 1933; Bhandari 1949; Vaishya 1953; Godbole *et al* 1966), *J. glandulifera* Linn. (Charak 1949), *J. multifida* Linn. (Bhandari 1949; Bhavmisra 1949a; Vaishya 1953), *Baliospermum montanum* Muell-Arg. (Sharma 1956), *Croton polyandrum*? (correct identity unknown) (Bhavmisra 1949b) and *C. tiglium* Linn. (Pendse and Iyenger 1961)—all belonging to the family Euphorbiaceae. Two other species implicated are *Chlorophytum tuberosum* Baker (Fam. Liliaceae) (Sushrut 1963) and *Salvinia cuculata* Roxb. (Fam. Salviniaceae) (Ray 1904). Of these, *J. glandulifera* has recently been recognised as the official 'Dravanti' by the Ayurvedic Pharmacopoeia Committee. However, the commercially available drug is rarely this species. In view of the above, a detailed study of the pharmacognostic characters of different implicated taxa was, therefore, considered worthwhile. The present communication being first of the series deals with pharmacognosy of *Jatropha curcas*. It is a shrub or a small tree. Originally a native of America it is now fully naturalised in India and is grown as a field barrier (Hooker 1879; Cooke 1958; Anonymous 1959; Chopra 1965).

Almost every part of it is credited with some medicinal properties (Waring 1968; Khory 1887; Watt 1908; Dutt 1922; Kirtikar and Basu 1933; Nadkarni 1954; Chopra *et al* 1958; Anonymous 1959; Bose *et al* 1961; Watt and Breyer 1962; Chopra 1965;

* NBRI Research Publication No. 241 (New Series)

Anonymous 1970; Dey 1973). Their chemical and biologically active constituents have been investigated (Dymock *et al* 1892; Felke 1914; Soliven 1928; Sukumaran and Menon 1943; Chopra *et al* 1956, 1969; Anonymous 1959; Sinha 1959; Watt and Breyer 1962; Torne and Joshi 1964; Mitra and Misra 1967; Mitra *et al* 1970; Subramanian *et al* 1971; Khafagy *et al* 1977; Charles and Babajide 1978). Rao and Malviya (1964) dealt with the distribution of laticifers and nature of latex and Miller and Webster (1962) compared cross-sections of petioles of *Jatropha* spp. with *Cnidoscolus* spp. However, no detailed pharmacognosy of the root, stem and leaf of *Jatropha curcas* has been carried out so far.

2. Material and methods

Fresh plant parts of *Jatropha curcas* were collected locally. Hand sections were used for histological studies. Microchemical tests for cell-contents and cell-wall structure were performed according to Johansen (1940) and Kay (1938). Isolated elements were studied after maceration with Schultz's fluid, the ash values and percentage extractives according to IP method (Anonymous 1966) and fluorescence characters according to Kokoski *et al* (1958). Behaviour of powdered drug on treatment with different chemical reagents was also noted. The palisade ratio, stomatal index, vein-islet and vein-termination numbers were determined according to the method given by Wallis (1967) and Hotch (1948) from leaves cleared by treating first with 10% nitric acid overnight followed by 80% chloral hydrate solution and heated for 30–60 min at about 100°C. Preliminary phytochemical studies and TLC patterns of the drug, were also carried out.

3. Observation

3.1 *Macroscopical characters*

3.1a *Root* (*figure* 1*A*): These are grey with longitudinal corrugations on the surface which in the older roots are long and anastomosing. Well developed, irregularly distributed lenticels appear globular on the younger parts but are elongated on the older regions. Usually the adjoining lenticels unite to form a transverse ridge on the root surface and after a certain stage of growth, forms transverse grooves instead. The roots on drying become very light and fracture short and uneven. Transversely smoothened surface shows light-cream coloured wood with distinct growth rings occupying the major part of the root and a considerably narrow bark of brown colour. These have a slightly bitter taste with pleasant and agreeable odour.

3.1b *Stem* (*figure* 1*A*): The young stem pieces are glandular tomentose. A piece of slightly mature stem has sparsely distributed small, oval lenticels on a smooth surface which on drying shows fine longitudinal striations. In a moderately thick stem the light grey or greenish white skin peels off easily as thin, translucent, papery flakes. In the older stem, however, the surface has sparse to dense tubercles as a result of the development of longitudinal rows of large corky lenticels. The surface is also longitudinally fissured or cracked along these lines. On drying, the stem shrinks considerably and its bark turns greyish to dull-brown. The smoothened transversely cut surface shows a white or creamy-white, soft, spongy wood feebly marked with a few growth rings occupying a major part of the stem. A brown narrow bark adheres to it

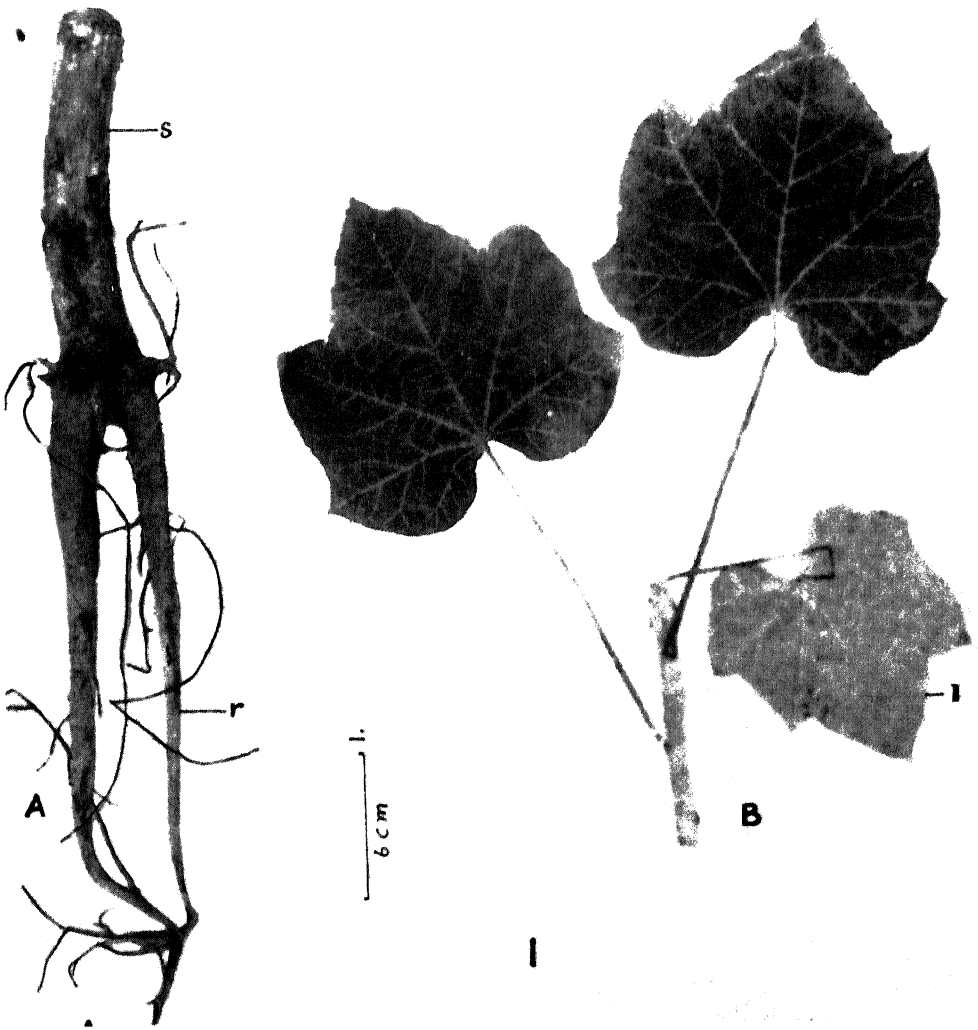


Figure 1. Macroscopic features of *J. curcas*. **A.** A portion of the root and the stem. **B.** A twig with leaves. (See p. 82 for explanation of abbreviations).

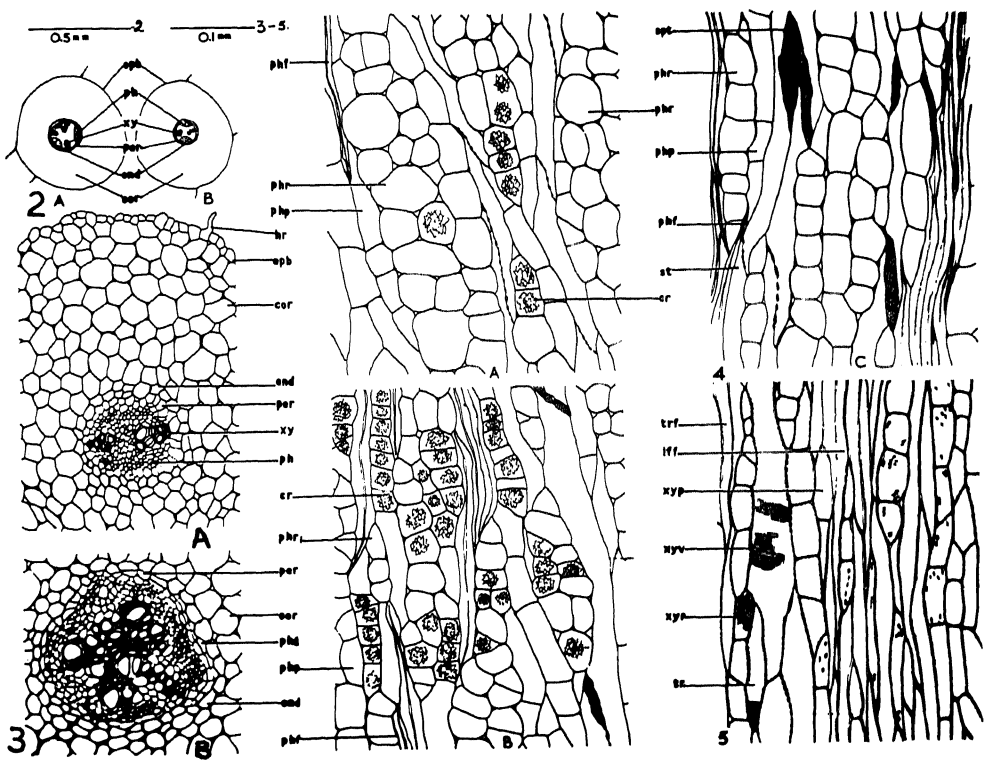
loosely. The stem has a characteristic disagreeable odour and taste when fresh but becomes odourless and almost tasteless on drying. The fracture in bark portion is fibrous and in wood short, splintery.

3.1c *Leaf* (*figure* 1*B*): The leaves are alternate or sub-opposite, elliptic or oblong-elliptic, obtuse or very often shortly cuspidate, measure 6·3–10 × 3·5–4·5 cm; glabrous, when fully grown pale dull glaucous green, base usually round, midrib prominent and pink, main nerves 6–10 and prominently raised above the surface on the abaxial side, venation between these reticulate; petiole 6–13 mm long. The leaves turn red before falling. The dried leaf is brittle, having astringent and bitter taste with no distinct odour.

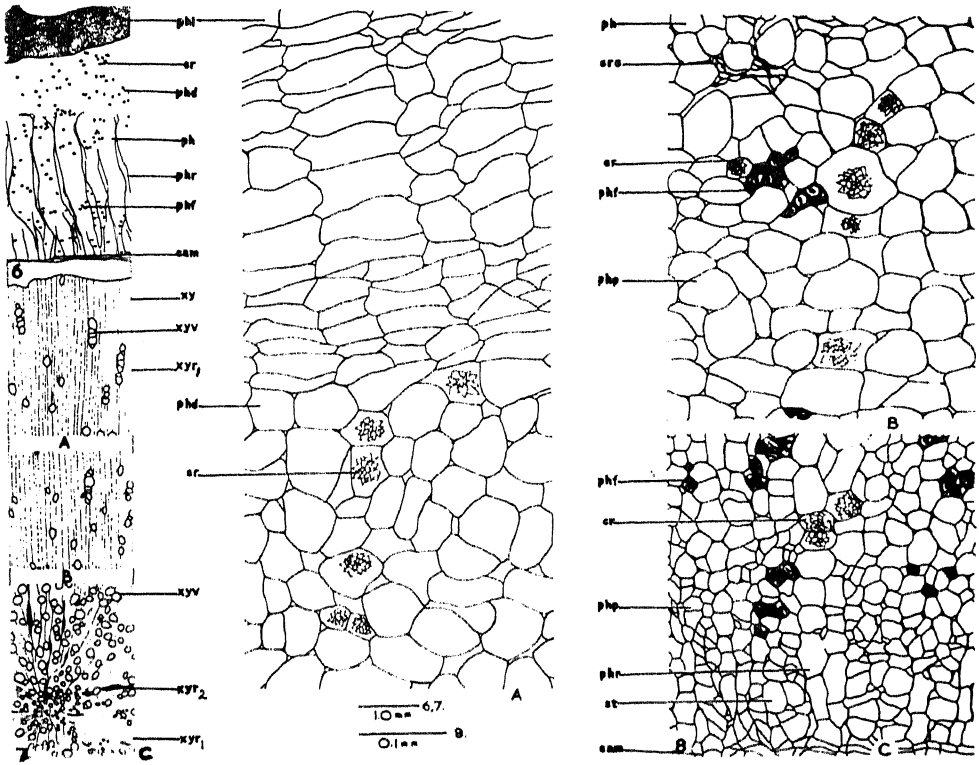
3.2 *Microscopic characters*

3.2a *Root* (*figures* 2–10): *Young root*: It shows a single layered epiblema consisting of cubical to more or less columnar cells (measure 8–16–21 × 8–12–16–21 μm), a number of which develop into root hairs (figures 2A–B, 3A). Below the epiblema is a 6 to 8 layered parenchymatous cortex with cells measuring 12–29–53–70 × 16–25–37–50 μm. The endodermis has barrel-shaped cells (measuring 12–16–24–29 × 8–12–16–21 μm) with distinct Casparian thickenings on the radial walls (figure 3A). A single layered pericycle below the endodermis encloses a di- to tetrarch stele having usual phloem and xylem elements (figures 2A–B, 3A).

A cork cambium arises in the pericycle and gives rise to the usual periderm cells (phellem and phelloderm) (figure 3B). The phellem cells in the younger roots are rectangular and flat. In older stages, however, as the lenticels develop, two types of cells are differentiated in this regions (i) the rectangular, flat and compactly arranged ones forming the closing cells (ii) somewhat spherical and loosely arranged which are the complementary cells. With the increase in secondary tissue, the cortical and endodermal cells collapse and small groups of phloem fibres are formed in the secondary phloem.



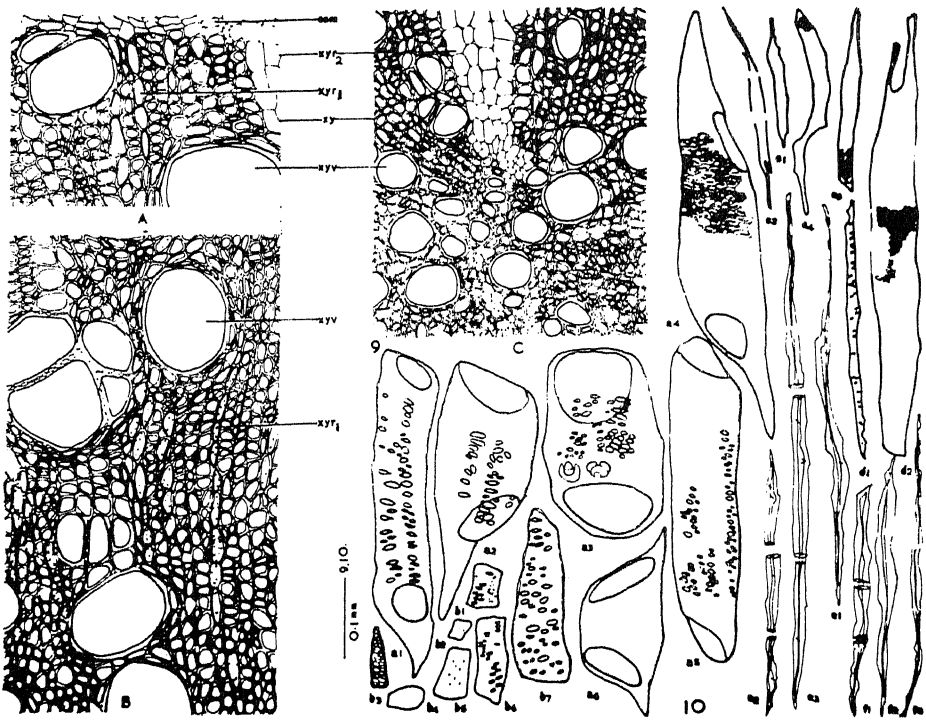
Figures 2–5. Microscopic characters of the root of *J. curcas*. **2.** Diagrammatic TS of young root. **A.** Showing triarch condition of xylem. **B.** Showing diarch condition of xylem. **3A.** Cellular details of a portion of figure 2B. **B.** Details in TS of a slightly mature root showing formation of phellogen in the pericyclic region. **4.** Details of mature root in LS through phloem. **A.** outer phloem, **B.** middle phloem, **C.** inner phloem. **5.** Details of mature root in LS through xylem. (See p. 82 for explanation of abbreviations)



Figures 6 8. Microscopic characters of root of *J. curcas*. **6.** Diagrammatic TS of a portion of the mature root-bark. **7A–C.** Diagrammatic TS of a portion of root-wood. **8.** Cellular details in TS of a portion of figure 6: **A.** A portion of the outer bark, **B.** middle bark, **C.** inner bark. (See p. 82 for explanation of abbreviations)

Mature root: The transection of bark in a mature root shows periderm consisting of 11–32 layers of phellem and a comparatively wider zone of phelloderm. Whereas the phellem cells are thin-walled, subserised and tangentially elongated (measure 45–57–102 –156 × 16–36–45–58 μm), the phelloderm cells vary in shape and size (28–61–102–144 × 20–49–74–107 μm) (figures 6, 8A). The secondary phloem occupies a considerably wider zone and consists of all the usual elements. In older barks some of the phloem elements collapse and get lignified to form ceratenchyma (figure 8B). The phloem fibres are either solitary or in small groups (figures 6, 8B–C). These are long (measure 16–20–33–41 × 495–1402–3252–3382 μm), thick-walled and lignified with tapering or truncated ends (figure 10 f1–f3). The sieve tubes are usually isodiametric (24–33–37 μm in TS) and have an oblique sieve plate on both, the end and the longitudinal walls (figures 4, 8C). Phloem parenchyma are variable in shape. The phloem rays are narrow (uni- and biseriate) and broad (3 to 8 seriate or so) and heterogeneous. The narrow rays are 1–9–17–33 cells high, 3–5 seriate ones 4–12–23–40 cells and 6–8 seriate rays 15–25–32–45 cells high (figures 4A–C). The cambium is 1 to 2 layered (figure 8C).

The xylem covers almost 3/4th of the root diameter and consist of the usual elements (figures 7, 9). The vessels are diffused porous, occur singly or in groups of 2 to 6. In macerations the vessel elements vary from cylindrical with peg like outgrowths at the ends to short wide and drum-shaped ones, measuring 56–59–165–198 × 148–338–



Figures 9 and 10. Microscopic characters of the root of *J. curcas*. **9.** Cellular details in TS of figure 7. **A.** A portion of the outer xylem, **B.** middle xylem, **C.** inner xylem **10.** Isolated elements of mature root: **a1–a6.** vessel elements, **b1–b7.** xylem parenchyma, **c1–c4.** tracheids, **d1–d2.** tracheid fibres, **e1–e3.** libriform fibres, **f1–f3.** phloem fibres. (See p. 82 for explanation of abbreviations).

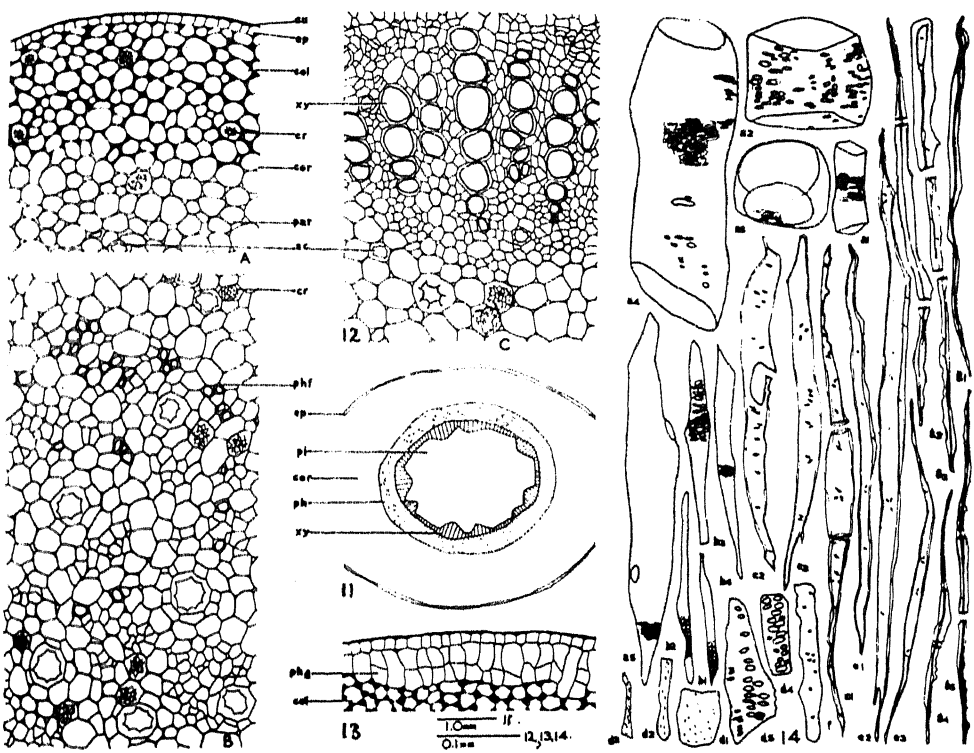
500–742 μm (figures 10, a1–a6). The perforation rims and bordered type of pits arranged alternately are quite distinct (figures 10, a4). However, opposite and scalariform pit arrangement is also observed (figure 10 a1–a3). The xylem parenchyma is diffused apotracheal (figures 9A–C). Some of the cells remain unlignified and have simple pits or reticulate thickenings on their walls. However, those adjacent to vessels are highly lignified and have distinct bordered pits similar to those in vessels (figure 10 b1–b7). The tracheids are long (20–33–41–50 × 297–417–570–760 μm) with tapering or branched extremeties and bordered pits on the walls (figure 10 c1–c4). Some of the fibre-tracheids are, however, extremely long and occasionally very wide (figure 10 d1–d2). The libriform xylem fibres measure 16–33–41–50 × 594–959–1270–1650 μm and usually occur in small groups distributed irregularly in the wood. These have tapering, truncated or bifurcated ends and appear like phloem fibres in macerations (figure 10 e1–e3). The xylem rays are heterogeneous. While the uni- and bi-seriate rays are 2–7–14–21 cells high, the multiseriate (3–4) ones are 6–10–14–17-celled (figure 5). Individual ray cells measure 24–33–45 × 20–66–98–152 μm in tangential sections.

3.2b *Stem* (*figures* 11–19): *Young stem*: It possesses a single layered epidermis with thin-walled, cubical to tangentially elongated cells (10–16–20–29 × 10–12–16 μm), covered externally with a thin cuticle (figures 11, 12A). The cortex consists of 6 to 8 layers

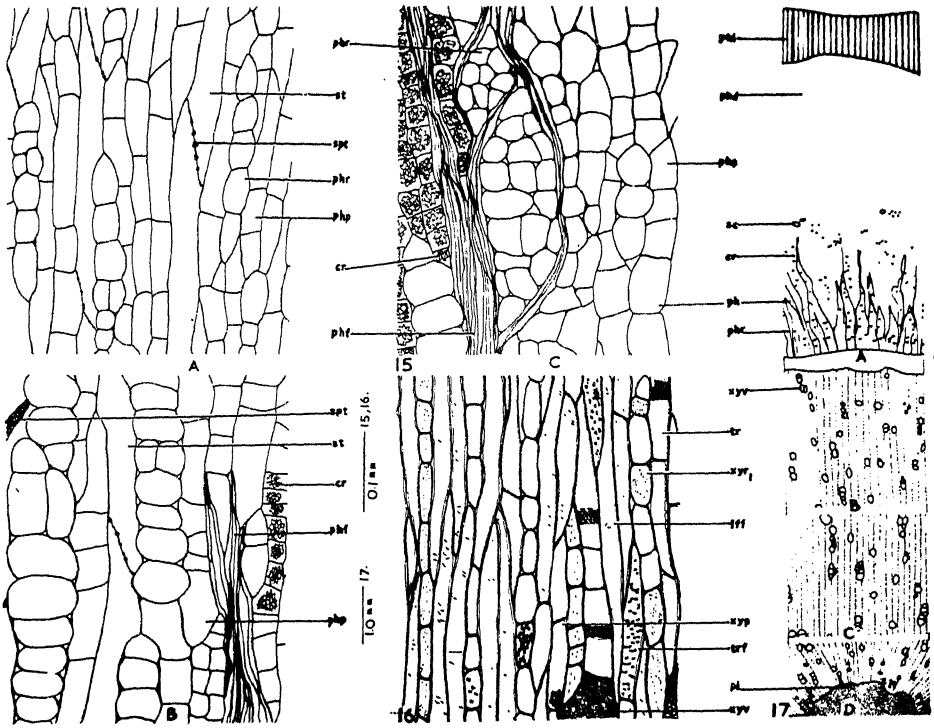
of collenchyma (measuring 12–19–33–41 × 16–33–45–53 μm) and the rest are parenchyma layers. There is no starch-sheath, nor is the pericycle discernible (figures 12A–B). The vascular tissue is represented by siphonostele having vascular bundles so closely placed that the xylem and phloem appear at first sight as closed circles (figures 11, 12 B, C). The pith has parenchymatous, isodiametric cells measuring 16–33–45–62 μm in diameter. Non-atriculated laticifers are found all over but become progressively wider towards the centre (in cortex: 12–16–29–41 μm, phloem: 25–41–47–57 μm and pith: 29–37–47–62 μm) (figures 12A–C).

The cork cambium, which arises in the outermost cortical layer, gives rise to phellem outside and phelloderm inside (figure 13). As the secondary growth progresses the epidermis gradually ruptures as flakes leaving the inner layers intact. In older bark some of the phloem cells collapse to form the ceratenchyma which may also get lignified.

Mature stem: The bark portion is distinguishable into an outer, middle and an inner zone in a mature stem. Of these the outer one is composed of a few layers of thin-walled, suberised cork cells (size: 12–21–29–41 × 29–41–57–74 μm) arranged in radial rows (figures 17A, 18A). The middle bark consists of somewhat tangentially elongated

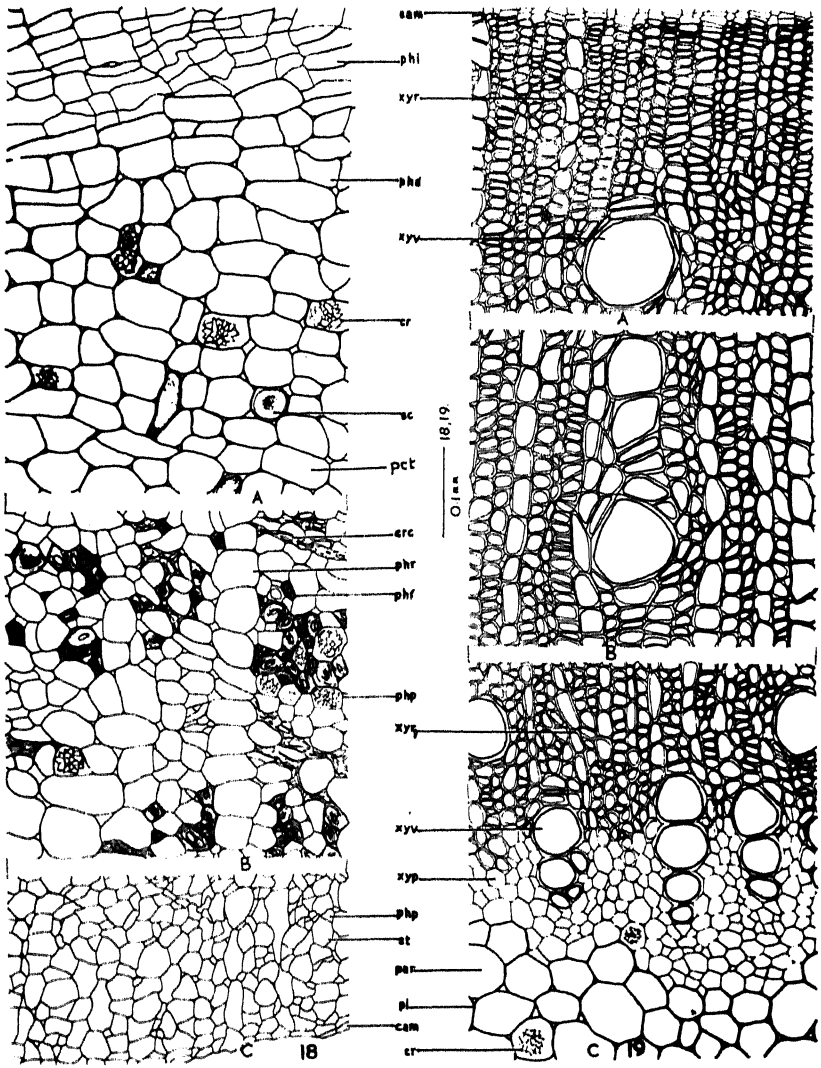


Figures 11–14. Microscopic characters of the stem of *J. curcas*. **11.** Diagrammatic TS of a portion of the young stem. **12.** A–C Cellular details in TS of portions of figure 11. **13.** Details in TS of a portion of slightly mature stem showing formation of phellogen in the outer most cortical layer. **14.** Isolated elements of the stem: **a1–a5.** vessel elements, **b1–b4.** tracheids, **c1–c3.** tracheid-fibres, **d1–d5.** xylem parenchyma, **e1–e3.** libriform fibres, **f.** tracheid-parenchyma, **g1–g5.** phloem fibres. (See p. 82 for explanation of abbreviations).



Figures 15–17. Microscopic characters of the stem of *J. curcas*. **15.** Details of mature stem in LS through phloem: **A.** a portion of the outer phloem, **B.** middle phloem, **C.** inner phloem. **16.** Details of mature stem in LS through xylem. **17.** Diagrammatic TS of a portion of the mature stem: **A.** bark, **B.** outer xylem, **C.** middle xylem, **D.** inner xylem and pith. (See p. 82 for explanation of abbreviations)

phelloderm cells which are comparatively smaller and narrower in the outer few layers. There is no definite demarcation between the phelloderm and the primary cortex (figures 17A, 18A). However, the laticifers in the latter zone are more frequent (figure 18A). The inner bark is composed of phloem tissue characterised by funnel shaped multiseriate rays which narrow towards the centre. The phloem, thus, appears arranged in wedge-shaped conical masses (figures 17A, 18B–C). This is again traversed by narrow usually uni- to biseriate rays which become comparatively wider in the middle region of the phloem (figure 17A). These are heterogeneous. While the narrow rays are 1–8–16–26 cell high (with individual cells measuring 21–33–49–66 × 21–41–62–74 μm) the broad rays are 9–15–25–44 or more cells high (with individual cells measuring 29–57–74–115 × 24–90–123 μm) (figures 15A–C). Phloem fibres may occur solitary or in radially arranged groups (figure 18B). These are 412–1320–2475–3597 μm long and 12–25–37 μm in diameter with tapering or truncated ends (figure 14g1–g4). The sieve tubes are crushed or collapsed at many places in the outer region (figure 18B). Their end walls have compound sieve plates arranged in a scalariform manner (figures 15A–B). Below the phloem are 1–2 layers of cambium as usual (figure 18C). The xylem, which occupies more than 3/4th parts of the stem diameter, shows a few feebly marked growth rings. The tissue is diffused porous. However, it is not unusual to find a narrow zone at the end of each growth ring with vessels sparsely distributed or completely absent. Vessels may

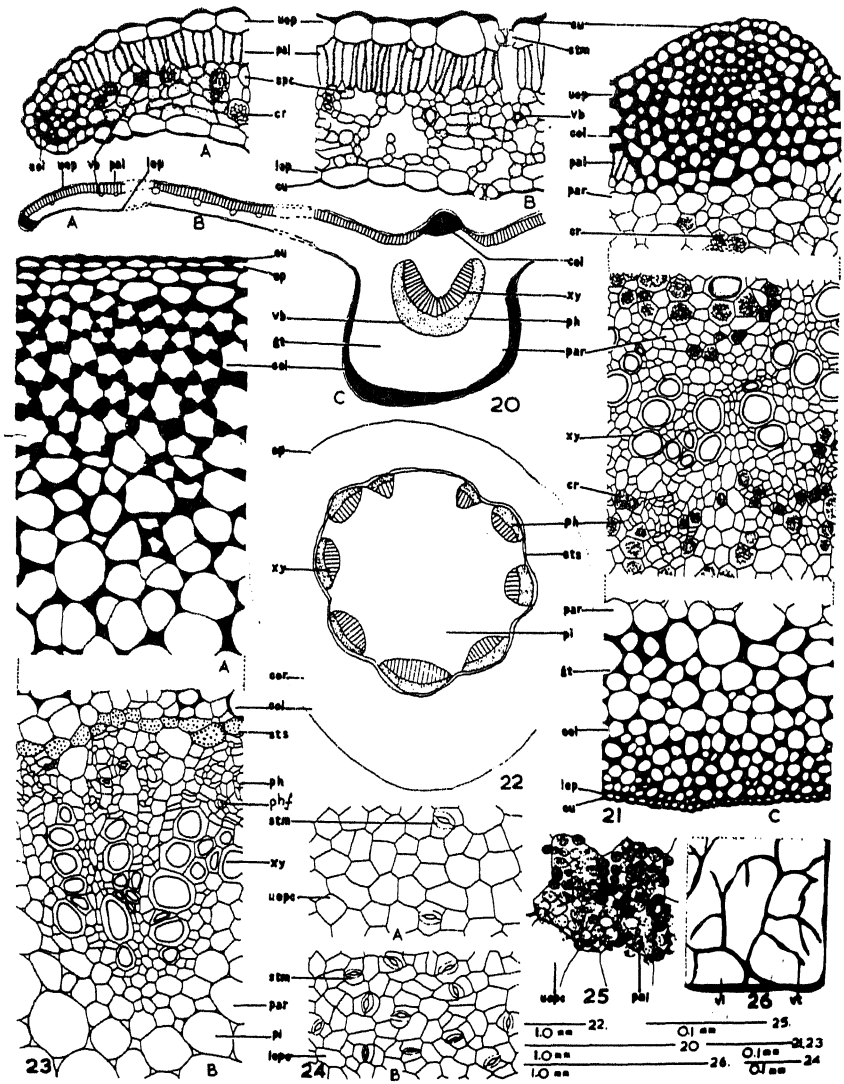


Figures 18 and 19. Microscopic characters of the stem of *J. curcas*. **18.** A–C Cellular details in TS of portions of figure 17A. **19.** Cellular details in TS of portions of figures 17B–D. (See p. 82 for explanation of abbreviations)

occur singly (solitary) or in groups of 2–12 arranged radially (figures 17B–D, 19A–C). These are 66–99–132–182 μm wide and 99–330–533–710 μm long, bordered pitted as those in root (figure 14a1–a5). Tracheids measure 16–25–33 × 164–471–545–808 μm (figure 14b1–b4), fiber tracheids 16–33–50 × 462–660–842–1188 μm (figure 14C1–C3) and libriform xylem fibres 16–25–33 × 990–1320–2962 μm (figure 14e1–e3). Xylem parenchyma (figure 14d1–d5), in all essential details, are similar to those in root. However, some of these are much elongated tracheid parenchyma (figure 14f). The xylem rays are heterogeneous and uni- or bi-seriate. In tangential section these are 1–10–22–45 cells long with individual cells measuring 16–32–48 × 33–48–98–130 μm (figure 16).

The pith has polyhedral, oval to almost isodiametric parenchyma (figure 19C).

3.2c *Leaf (figures* 20–26): *Petiole*: Its transection is plano-convex to almost circular in outline (figure 22). The epidermis has a moderately thick cuticle on the outside and is composed of narrow, tangentially elongated cells measuring 4–6–12 × 8–20–29–41 μm (figure 23A). It is followed by 14–17 layers of collenchyma (measure 20–23–49–70 × 37–57–78 μm) and a few layers of parenchymatous cells measuring 20–37–74–111 × 24–66–115 μm. A single layered starch-sheath is present but no pericycle is discernible



Figures 20–26. Microscopic characters of the leaf of *J. curcas*. **20.** Diagrammatic TS of a portion of lamina: **A.** marginal lamina, **B.** middle lamina, **C.** mid-rib. **21.** Cellular details of figure 20. **A.** marginal lamina, **B.** middle lamina, **C.** a portion of the mid-rib. **22.** Diagrammatic TS of a portion of basal end of the petiole. **23.** Cellular details of a portion of figure 22. **24.** Epidermis of lamina in surface view: **A.** upper epidermal cells and stomata, **B.** lower epidermal cells and stomata. **25.** Palisade cells in surface view. **26.** Vein-islets and vein-terminations of lamina in surface view. (See p. 82 for explanation of abbreviations)

(figure 23B). A TS near the basal end of the petiole shows a eustele of 9 collateral vascular bundles (figure 22), which coalesce to form a large semicircular vascular arc and a small separate bundle on the adaxial side. Near the apex all the bundles of the petiole separate out again to enter the lamina at different levels. The phloem besides its usual cells consists of phloem fibres also (diameter 20–23–33–41 μm) and the xylem is composed of vessels, tracheids, xylem parenchyma and xylem rays arranged radially (figure 23B). The central ground tissue has thin-walled isodiametric to polygonal cells.

Leaf: The TS of the lamina presents a dorsiventral structure (figure 20). The epidermal cells on both the surfaces are, as usual, tangentially elongated and have a fairly thick cuticle (figures 21A–B). The upper epidermal cells, however, are somewhat larger (measure 20–28–33–41 × 20–33–53–82 μm) than those of the lower surface (measure 20–25–27–33 × 18–29–32–54 μm) and in a surface view appear polyhedral with comparatively more straight walls than those of the lower epidermal cells (figures 24A–B). Both paracytic and anisocytic stomata (measure 20–25–33 × 33–37–45 μm) occur on two surfaces but are more frequent on the abaxial side (figures 24A–B).

The mesophyll consists of a single layered palisade and a few layers of spongy parenchyma as usual (figures 20A–B). The palisade cells are columner (8–10–12 × 20–31–41–54 μm) and closely packed. A few mesophyll cells develop distinctly dense cell-contents and primary pits on their walls. A few idioblasts containing crystals are present in this region (figures 21A–B). At the margins, the mesophyll cells are replaced by thick-walled collenchymatous cells (8–12–17 × 8–12–19 μm). The small veins are embedded and surrounded by a bundle sheath while the larger ones are usually transcurrent. The former may have both phloem and xylem or just 1 or 2 tracheary elements (figures 21A–B). The primary veins are prominently raised above the surface on the abaxial and slightly on the adaxial side (figure 20C). The epidermal cells over the veins are similar to but somewhat smaller than those on the margins (measure 8–12–16–33 × 8–12–21–37 μm) (figure 21C). These are followed by several layers of collenchyma (measuring 12–20–33–41 × 12–25–37–45 μm) on the adaxial side. Below these are a few layers of parenchyma (measure 16–24–33–58 μm in diam.). A small patch of collenchyma occur in the adaxial bulge as well. The starch sheath is not discernible and unlike the petiole there is only a single crescent-shaped median vascular patch with the xylem facing the upper and the phloem the lower side (figures 20C, 21C). The laticifers (20–49 μm in diam.) besides being associated with veins, also creep along the palisade and spongy mesophyll.

3.3 *Quantitative histology*

Quantitative determination of the stomatal index (figures 24A–B), palisade-ratio (figure 25), vein-islet and vein-termination number (figure 26) of the leaf was also carried out and the results are presented in table 1.

3.4 *Cell-contents*

Simple starch grains occur in parenchymatous cells of the bark of both the root and the stem and their xylem rays and also in some of the cells of the leaf petiole. The grains are circular, oval with a central linear hilum and measure 6–12–19 × 4–12–20 μm. Rosette crystals of calcium oxalate (measuring 12–28–41–57 × 16–24–50–83 μm) are found frequently in the parenchymatous cells of the cortex and pith of young stem,

Table 1. Quantitative histological values of leaf of *Jatropha curcas*.

Values	Location*			Mean Value	Range of variation
	Base	Middle	Apex		
Stomatal index					
Upper surface	6·3–7·25–8·9	4·36–6·2–9·6	7·4–7·7–7·9	7·33	3–12
Lower surface	8·8–12·7–13·1	11·2–11·3–12	12·8–12·9–13·8	12·09	7–14
Palisade-ratio	14·5–17–24·2	16·2–21·6–22·5	17·3–20–21·6	19·47	13–26
Vein-islet number	1–1·6–2·3	1–2–2·6	2·3–4–5·3	2·44	0–6
Vein-termination number	15–15·6–16·3	15–15·6–16·6	16–18·6–37	18·51	12–42

* Values represent mean of 9 readings.

phelloderm and phloem tissue of barks of both root and stem and also in the laminar ground tissue and phloem of leaf. However, in the petiole these are present sparsely. A dark brown content occurs in non-articulated, branched laticifers of all the parts of the plant. Steroids, reducing sugars, polysaccharides/glycosides and resins are present in various extractives (table 2).

3.5 *Powder study*

The leaf powder is yellowish green, has a slightly astringent and bitter taste and no perceptible odour. A microscopic examination reveals small fragments of lamina with epidermis having anisocytic and paracytic stomata, mesophyll cells, broken parts of tracheary elements and laticifers associated with mesophyll and phloem tissue.

The stem powder is grey in colour and has no perceptible odour and taste. A microscopic examination shows small groups of cork cells and parenchyma, broken parts of laticifers filled with dark brown contents, isolated and small groups of broken fibres, tracheids and vessels.

The root powder, however, is light brown, has a slight bitter taste and a pleasant odour. Microscopically it is similar to the stem powder.

Simple starch grains and calcium-oxalate crystals are found in the powder. The behaviour of the different powders on treatment with different chemical reagents is shown in table 3. Fluorescence characters of different powders were also studied and are recorded in table 4. The percentage-extractives and ash-values are given in table 5.

3.6 *Preliminary phytochemical studies*

Three samples each of powdered leaf, stem and root (20 g) were soxhlated successively with different solvents and the physical appearance of the extractives and their average yield percentage were noted (table 2). These were further screened for plant bases, glycosides/polysaccharides, reducing sugars, saponins, flavonoids, sterols and tannins using the usual methods. The results obtained are summarised in table 2. The different extractives were also subjected to thin-layer chromatography on silicagel G following the usual procedure (figures 27–32).

With benzene-chloroform-acetone (35·5 : 7·5 : 5) as solvent system the petroleum-ether extractive gave as many as 7 distinguishable spots (hRf 2·7, 8·7, 12·1, 37, 51·3, 60·1, 86·4) in the root, 8 in the stem (hRf 3·2, 7·4, 16·8, 19·5, 35·1, 45·9, 81·7, 97·4) and 8 in the

Table 2. Summary of preliminary phytochemical examination of *J. curcas*.

Plant part.	Observation	Pet ether (60–80°) extract	Benzene extract	Chloroform extract	Acetone extract	Alcohol extract	Water extract
	Physical appearance	yellow	yellow	yellow	pale yellow	light brown	dark brown
	Average yield percentage (w/w)	1·202	0·63	0·184	0·27	2·484	4·968
	Sterols	–	–	–	–	–	–
	Bases	–	–	–	–	–	–
	Saponins	–	–	–	–	–	–
Root	Glycosides/polysaccharides	–	–	–	+	+	+
	Flavonoids	–	–	–	–	–	–
	Reducing sugars	–	–	+	+	+	–
	Resins	–	–	+	+	–	–
	Tannins	–	–	–	–	–	–
	Physical appearance	light yellowish green	light yellowish green	light yellowish green	brown	brown	dirty brown
	Average yield percentage (w/w)	0·55	0·184	0·222	0·414	8·23	8·012
	Sterol	+	+	–	–	–	–
	Bases	–	–	–	–	–	–
	Saponins	–	–	–	–	–	–
	Glycoside/polysaccharides	–	–	–	+	+	+
Stem	Reducing sugars	–	–	+	+	+	–
	Flavonoids	–	–	–	–	–	–
	Resins	–	–	+	+	+	–
	Tannins	–	–	–	–	–	–
	Physical appearance	yellowish green	dark yellowish green	dirty yellowish green	dirty orange	brown	reddish brown
	Average yield percentage (w/w)	3·324	1·34	0·938	1·83	7·4	15·98
	Sterols	+	+	+	–	–	–
	Bases	–	–	–	–	–	–
Leaf	Saponins	–	–	–	–	–	–
	Glycoside/polysaccharides	–	–	–	+	+	+
	Reducing sugars	–	–	+	+	+	+
	Flavonoids	–	–	–	–	–	–
	Resins	–	–	+	+	+	–
	Tannins	–	–	–	–	–	–

The total yield was 9·74, 17·612 and 30·606 in the root, stem and leaf respectively.

leaf (hRf 3·2, 8, 14·1, 23·5, 40·5, 46·6, 77·7, 87·1) besides a few trailings (figure 27). Of these, three spots in stem (hRf 3·2, 7·4, 45·9) and leaf (hRf 3·2, 8, 46·6) were more or less comparable. Again, the spot at 86·4 in the root seems similar to the one in the leaf at 87·1.

With the developing solvent system benzene-chloroform-acetone (2:7:1) the benzene extractives separates into 1 distinct spot (hRf 89·7) and two elongated trails at

Table 3. Behaviour of powdered root, stem and leaf of *J. curcas* on treatment with different chemical reagents.

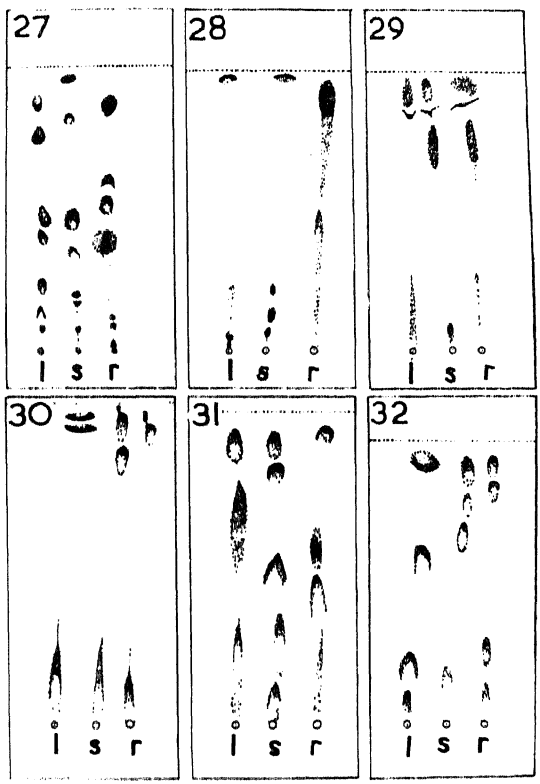
Reagents	Behaviour of powder		
	Root	Stem	Leaf
Picric acid (saturated soln.)	Dirty orange	Dirty yellow	Dull-yellow
Nitric acid (Sp. gr. 1·42)	Dull-brown	Reddish brown	Dirty orange
Hydrochloric acid (Sp. gr. 1·66)	Greyish brown	Greyish brown	Blackish brown
Sulphuric acid (80% soln.)	Olive-brown	Blackish brown	Olive-green
Acetic acid	Light greyish brown	Buff-brown	No change
Lactic acid	Light greyish brown	Buff-brown	Yellowish grey
1N NaOH (aq. soln.)	Chocolate-brown	Chocolate-brown	Brown
Iodine(aq. soln.)	Light brown	Brown	Grey
Ferric chloride (5% aq. soln.)	Grey	Grey	Dirty yellowish green
Antimony trichloride in $HClO_4$ (saturated soln.)	No change	No change	Moderate olive-green under microscope some red stained tissues are observed scattered among yellowish green tissue.

Table 4. Fluorescence analysis of root, stem and leaf of *J. curcas*.

Treatment	Fluorescence		
	Root	Stem	Leaf
Drug powder mounted in nitrocellulose in amylacetate	Dark green	Light green	Green
Drug powder treated with 1N NaOH in methanol	Bright yellowish green	Dark green	Blackish green
Drug powder treated with 1N NaOH in methanol and mounted in nitrocellulose in amylacetate	Olive-green	Parrot-green	Dark green
Drug powder treated with 1N HCl	Pale-green	Yellowish green	Yellowish green
Drug powder treated with 1 N HCl and mounted in nitrocellulose in amylacetate	Pale-green	Light yellowish green	Bright green
Drug powder treated with 1N NaOH (aq. soln.)	Green	Bright green	Moderate olive-green
Drug powder treated with 1N NaOH (aq. soln.) and mounted in nitrocellulose in amylacetate	Dark green	Dirty green	Dark olive-green
Drug powder treated with 50% HNO_3	Yellowish green	Yellowish green	Yellowish green
Drug powder treated with 50% H_2SO_4	Dark green	Green	Green
Drug powder as such	White with greenish tinge	Pale-green	Green

Table 5. Ash values and percentage extractives (IP) of root, stem and leaf of *J. curcas*.

Values	Plant-part (%)		
	Root	Stem	Leaf
Total ash	8·91	8·01	10·55
Acid insol. ash	1·5	0·51	2·091
Water sol. extractive	9·36	12·08	18·65
Alcohol soln. extractive	4·07	2·752	10·89



Figures 27 32. TLC pattern of various extractives of *J. curcas*. **27.** Petro. ether extractive. **28.** Benzene extractive. **29.** Chloroform extractive. **30.** Acetone extractive. **31.** Alcohol extractive. **32.** Water extractive. (See p. 82 for explanation of abbreviations)

hRf 25·3 and 66·4 in the root; 4 distinct ones (hRf, 6·8, 12·3, 21·3, 97·4) and 1 small trail (at hRf 2·7) in the stem and 3 distinct ones (hRf 2, 6·1, 97·2) and 2 trails (at hRf 12·3, 20·5) in the leaf (figure 28). Spots with hRf 2·7, 6·8, 12·8 and 97·4 respectively in the stem appear to be similar with spots having hRf 2, 6·1, 12·3, 97·2 in the leaf. However, the root extract did not give satisfactory results with the same solvent system.

Again, the chloroform extractive resolved into 2 long trailings (hRf 14·3, 61·6) and 3

distinct spots (hRf 73·2, 87·6, 93·8) in the root; 1 small trailing (hRf 4·7) and 3 distinct spots (hRf 73·2, 84·9, 93·1) in the stem and 1 trailing (hRf 1·3) and 2 distinct spots (hRf 84·9, 92·4) in leaf with the solvent system *n*-butanol-acetone-acetic acid-water (20:25:6:4) (figure 29). All, but one spot in both the stem (hRf 4·7) and the root (hRf 1·6), are more or less comparable.

The acetone extractives resolved into 1 trailing spot at hRf 15·2 and 2 spots at hRf 79·8, 96·3 in the root; 1 trailing spot at 15 and 3 distinct spots (hRf 83·1, 92·1, 98·7) in the stem; 1 long trailing (hRf 15·6) and 2 distinct spots at hRf 92·7, 95·1 in leaf with the solvent system *n*-propanol-methanol-acetic acid-water (20:25:1:5) (figure 30).

The alcoholic extract of the root separates into 4 spots (hRf 15·4 trailing, 43·2, 56·7, 91·3) with the solvent system *n*-propanol-methanol-water (25:20:5), similar extractives of the stem and the leaf with same solvent system resolved in 5 spots (hRf 6·7 trailing, 30·8 trailing, 51·8, 80·2, 90·1) and 3 spots (hRf 21·6, 61·7, 89·5) respectively (figure 31). Of these, only one spot of the root, stem and leaf, respectively, having hRf 91·3, 90·1 and 89·5 seems to be similar.

Water soluble extractives of all the three plant parts distinctly resolved into 4 spots having hRf 14·3, 26, 82·1, 91·7 in the root; 10·2, 63, 78, 91 in the stem and 6·1, 20·5, 58·9, 92·4 in the leaf (figure 32). Of these, one spot in all the three extractives (hRf 91·7 in root, 91 in stem, 92·4 in leaf) appears comparable.

4. Discussion and conclusions

'Dravanti' is one of the several drugs of the traditional system of medicine, the identity of which is still unsettled. Besides *Jatropha curcas* several other species (mentioned earlier) have been implicated in 'Dravanti'. Only a detailed study of the various characters of all the implicated taxa can clear up the prevailing confusion.

Pendse and Iyenger (1961) have already provided certain diagnostic characters of the root of *Croton tiglium*. From the present observation it is evident that certain characters such as the presence of 9 leaf traces in the basal part of the petiole; lacunose mesophyll traversed by both transcurrent and embedded veins with one cell thick bundle-sheath; presence of mixed anisocytic and paracytic stomata and stomatal indices of 7·3 and 12 for the upper and lower surfaces respectively; presence of funnel-shaped multiseriate phloem rays demarcating phloem into wedge-shaped masses composed of fibres and ceratenchyma besides other phloem elements in both the root and the stem; diffused porous wood with diffused apotracheal parenchyma and 1–3 seriate xylem rays, alongwith certain physico-chemical constants and fluorescence characters can provide useful parameters for differentiating *Jatropha curcas* from other implicated taxa.

Acknowledgement

The author is extremely grateful to Director, National Botanical Research Institute for facilities.

References

Anonymous 1959 *The Wealth of India (raw materials)* (New Delhi: CSIR) Vol 5 pp. 293
Anonymous 1966 *Pharmacopoeia of India* (New Delhi: Government of India) pp. 947

Anonymous 1970 *Medicinal plants of Nepal* (Thapathali Kathmandu: His Majesty's Govt. of Nepal) pp. 135
Bhandari C R 1949 *Banaushadhi Chandrodaya* 2nd edn (Bhanpura-Indore: Gyan Mandir), vol 5 1130
Bhavmisra 1949a *Bhava Prakash* (ed.) Pt. Brahma Shankar Misra 2nd edn (Banaras: Chowkhamba Sanskrit Series Office), Part I 346–347
Bhavmisra 1949b *Bhava Prakash*, (ed.) Shri Vishwanath Dwivedi 2nd edn (Banaras: Motial-Banarasidas) pp. 224
Bose B C, Sepaha G C, Saffi A Q and Vargiya R V 1961 Observation on the pharmacological action of *Jatropha curcas; Arch. Int. Pharmacodyn.* **130** 28–31
Charak 1949 *The Carak Samhita* Delux Edn (Jamnagar: Sri Gulab Kunverba Ayurvedic Society), part 6: *Sutra*- 1/78; 2/10; 25/49; 30/64; *Viman*- 7/26; 8/136; *Chikitsa*- 7/124; 13/154; 23/241; 27/52; *Kalp*- 1/6; 11/13; 17; 12/1, 3, 12, 14, 18, 23, 40; *Siddh*-10/25; 11/25
Charles D H and Babajide O O 1978 Non-polar constituents *of J. curcas; Lloydia* **41** 161–165
Chopra R N 1965 *Poisonous plants of India* (New Delhi: ICAR) vol. 2 792–794
Chopra R N, Chopra I C, Handa K L and Kapoor L D 1958 *Chopra's indigenous drugs of India* 2nd edn (Calcutta: U N Dhur and Sons) pp. 587, 605 and 676
Chopra R N, Chopra I C and Verma B S 1969 *Supplement to glossary of Indian medicinal plants* (New Delhi: CSIR) pp. 48
Chopra R N, Nayar S L and Chopra I C 1956 *Glossary of Indian medicinal plants.* (New Delhi: CSIR) pp. 145
Cooke T 1958 *Flora of Bombay* Reprinted edn (Calcutta: BSI) vol. 3 pp. 93
Dey K L 1973 *The indigenous drugs of India* 2nd edn (New Delhi: The Chronica Botanica) pp. 169
Dymock W, Warden C J H and Hooper D 1892 *Pharmacographia Indica* (Calcutta: Thacker-Spink and Co.) vol. 6 pp. 274
Dutt U C 1922 *The materia medica of the Hindus* (Calcutta: Madan Gopal Dass) pp. 303
Felke E 1914 The poisonous principles of the seeds of *Jatropha curcas; Landw. Versuchsw* **82** 427; vide *Chem. Abstr.* 1914 **8** 531
Godbole S R, Pendse G S and Badekar V A 1966 *Glossary of vegetable drugs in Vagbhatt* (Poona: IDAR) pp. 118
Hooker J D 1879 *Flora of British India* Reprinted 1954 edn (Kent: L. Reeve and Co. Ltd.), vol. 5 pp. 383
Hotch J H 1948 Fifty-years of quantitative microscopy in pharmacognosy; *Econ. Bot.* **2** 111–116
Johansen D A 1940 *Plant microtechnique* (New York and London: McGraw Hill) pp. 182–203
Kay A L 1938 *Microscopical studies of drugs* (London: Bailliere Tindall and Cox) pp. 18
Khafagy S M, Mohmed Y A, Abel-Salam N A and Mohamoud Z F 1977 Phytochemical study of *Jatropha curcas; Planta Med.* **31** 274
Khory R N 1887 *The Bombay materia medica and their therapeutics* (Bombay: Ranian's Union Press) pp. 490
Kirtikar K R and Basu B D 1933 *Indian medicinal plants*, 2nd edn (Allahabad: Lalit Mohan Basu) Vol. 3 2244–2247
Kokoski C J, Kokoski R J and Slama F J 1958 Fluorescence of powdered vegetable drugs under ultraviolet; *J. Am. Pharm. Assoc. (Sci. End.)* **47** 715–17
Miller K and Webster G 1962 Systematic position of *Cnidoscolus* and *Jatropha; Brittanica* **14** 174–180
Mitra C R and Misra P S 1967 Amino acids of processed seed meal proteins; *J. Agric. Food. Chem.* **15** 697–700
Mitra C R, Bhatnagar S C and Sinha M L 1970 Chemical examination of *Jatropha curcas; Indian J. Chem.* **8** 1047
Nadkarni A K 1954 *Dr. Nadkarni's Indian materia medica* (Bombay: Popular Book Depot) vol. 1 pp. 705
Narahari 1933 *Raj Nighantu* 2nd edn (Calcutta: Siddheshwar Press) pp. 92
Pendse G S and Iyenger M A 1961 *Studies in Indian medicinal plants used in Ayurveda-Cathartics* (Poona: IDRA) pp. 44–46
Ray P C 1904 *A History of Hindu chemistry* Vol. 1 38
Rao A R and Malviya M 1964 On the latex cell and latex of *Jatropha; Proc. Indian Acad. Sci.* **B60** 95–106
Sharma P V 1956 *Dravya Guna Vigyan* (Banaras: Chowkhamba Vidya Bhawan) vols 2 and 3 pp. 358
Sinha A 1959 Chemical examination of the seeds of *Jatropha curcas; J. Proc. Inst. Chem.* **31** 213–217
Soliven F A 1928 The seeds and oils of *Jatropha curcas; Philippine Agric.* **16** 587–596
Subramanian S S, Nagrajan S and Sulochana N 1971 Flavonoid of some Euphorbiaceous plants; *Phytochemistry* **10** 2548–2549
Sukumaran K A R and Menon K N 1943 Fixed oil from *Jatropha curcas* Linn; *Proc. Indian Acad. Sci.* **A18** 160–163
Sushrut 1952 *Sushrut samhita*, (ed.) Shambhunath Pandey (Kanpur: Saraswati Pustakalaya) *Su*-45/124, pp. 144
Sushrut 1963 *Sushrut Samhita*, (ed.) Kunja Lal (Varanasi: Chowkhamba Sanskrit Series) vol 3 pp. 69

Torne S C and Joshi G V 1964 Mineral elements in various parts of *Jatropha curcas* Linn. during flowering I; *Biol. Sci.* 7 53–63

Vagbhatt 1950 *Astang Hridaya* (Banaras: Chowkhamba Sanskrit Series Office) *Su*-15/1,45; *Chik.* 15/17; 19/86; *Ka*-5/39, 49, 51; *Ut* 2/51; 3/13

Vaishya S 1953 *Shaligram Nighantu Bhushanam* (Bombay: Khemraj Krishandas) pp. 397

Wallis T E 1967 *Text book of pharmacognosy*; 5th edn (London: J and A Churchill Ltd.) pp. 113–116

Waring J 1968 *Pharmacopoeia of India* (London: W. H. Allen and Co.) pp. 203

Watt G 1908 *Commercial products of India* pp. 699

Watt J M and Breyer-Brandwijk M G 1962 *The medicinal and poisonous plants of Southern and Eastern Africa* 2nd edn (Edinburgh and London: E and S Livingstone Ltd.) pp. 420–422

Abbreviations: cam, cambium; col, collenchyma; cor, cortex; cr, crystal; crc, ceratenchyma; cu, cuticle; end, endodermis; ep, epidermis; epb, epiblema; gt, ground tissue; hr, root-hair; l, leaf; lep, lower epidermis; lepc, lower epidermal cell; lff, libriform fibre; pal, palisade cells; par, parenchyma; pct, primary cortex; per, pericycle; ph, phloem; phd, phelloderm; phf, phloem fibre; phg, phellogen; phl, phellem; php, phloem parenchyma; phr, phloem ray; phr1, narrow phloem ray; pi, pith; r, root; s, stem; sc, secretory canal (laticifer); spc, spongy parenchyma; spt, sieve plate; st, sieve tube; stm, stoma; sts, starch-sheath; tr, tracheid; trf, tracheid fibre; uep, upper epidermis; uepc, upper epidermal cell; vb, vascular bundle; vi, vein-islet; vt, vein-termination; xy, xylem; xyp, xylem parenchyma; xyr, xylem ray; xyr1, narrow xylem ray; xyr2 broad xylem ray; xyv, xylem vessel.

STATEMENT ABOUT OWNERSHIP AND OTHER PARTICULARS ABOUT PROCEEDINGS OF THE INDIAN ACADEMY OF SCIENCES

(Plant Sciences)

1. Place of Publication	..	Bangalore
2. Periodicity of Publication	..	6 issues a Year
3. Printer's Name	..	C N R Rao Indian Academy of Sciences Bangalore 560 080
4. and 5. Publisher and Editor	..	C N R Rao
6. Nationality	..	Indian
7. Address	..	Indian Academy of Sciences P B No. 8005 Bangalore 560 080
8. Name and Address of the Owner	..	Indian Academy of Sciences Bangalore 560 080

I, C N R Rao, hereby declare that the particulars given above are true to the best of my knowledge.

Dated 1st March 1985

C N R Rao
Signature of Publisher

Dedicated to C V Subramanian
on his
60th Birthday

C. V. Subramanian (*born 11 August 1924*)

FOREWORD

Prof. C. V. Subramanian has been closely associated with the Indian Academy of Sciences for the last three decades. A scientist of eminence, C. V. Subramanian has endeared himself to all by his charming manners and his insistence on quality in scientific research. As Chairman of the Editorial Board of *Proceedings* (*Plant Sciences*) he was greatly concerned with the maintenance and improvement of the journal. It is most appropriate therefore that a special volume of the journal is brought out to commemorate his sixtieth birthday.

On behalf of the Academy, our thanks go to all the authors who most willingly agreed to contribute to the volume. To C. V. Subramanian, our best wishes for many more happy and fruitful years.

Bangalore
6 April 1985

S. Ramaseshan
President
Indian Academy of Sciences

DEDICATION

This Festschrift to Professor C. V. Subramanian has been brought out to mark his sixtieth birthday and to greet him on the occasion. Elected a Fellow when he was barely 31, Subramanian has been closely associated with the Academy as Chairman, Editorial Board for Plant Sciences since 1976 and as a Vice-President since 1977.

Born in Ernakulam, Subramanian is the son of C. M. Venkatachalier, a leading lawyer. Having lost his father at an early age, he was brought up by his mother. He had his schooling and early education in Ernakulam and then joined the Presidency College, Madras from where he graduated with an Honours degree in Botany with a first class first in the University in 1944. In the same year he joined the University Botany Laboratory, Madras and came under the tutelage of Professor T. S. Sadasivan. Subramanian was greatly stimulated by S. D. Garrett's *Root Disease Fungi* and Selman Waksman's *Microbial Antagonism and Antibiotic Substances*. He obtained his Ph.D. (1947) and D.Sc. (1957) degrees from the University of Madras. His first appointment was with the University of Madras in 1951. He was the first to hold the Chair in Plant Pathology at the Indian Agricultural Research Institute, New Delhi (1958–60) and the Chair in Botany at the University of Rajasthan (1960–64) at Jodhpur and then at Jaipur. He has been Professor (from 1964) and later Director (since 1973), Centre for Advanced Study in Botany, University of Madras where he has built up an active school of research in fungal taxonomy and ecology.

Subramanian has been engaged in research and teaching Mycology and Plant Pathology for more than three decades. His chief original research contributions are in the field of taxonomy and biology of fungi. His early interest in soil mycology led to his pioneering work on the autecology of *Fusarium oxysporum* f. sp. *vasinfectum*, the cotton wilt pathogen whose positive susceptibility to microbial antagonism in soil and efficient mode of perennation as a competitive colonizer on plant debris were demonstrated and, for this reason, was classified as a *soil inhabitant*. In later work, Subramanian elucidated the occurrence and status of Fusaria of Indian soils in a series of papers. He has continued his interest in fungal ecology: and over a period of years he has made significant contributions to our knowledge of fungal ecology, especially coprophilous, marine, and soil and litter fungi.

His most outstanding contributions are in the field of Hyphomycetology, in which he is a world authority. His painstaking work of over two decades culminated in the publication of a monograph entitled "HYPHOMYCETES" by the Indian Council of Agricultural Research in 1971. He subsequently refined the earlier concepts on conidiogenesis and also presented a new terminology pertaining to conidiogenesis. He examined in detail the origin and nature of conidial chains and discussed the relationship of phialidic Hyphomycetes to their teleomorphs. The recently published book, HYPHOMYCETES : TAXONOMY AND BIOLOGY (1983 Academic Press, London) is his *magnum opus*, and it reflects his depth of understanding, comprehensive experience and critical analysis.

His extensive collections in South India have brought to light a large number of

microfungi, interesting or new to science. Much of the work on microfungi from the forests of the Western Ghats is yet to be published. Several fungi of potential use in biotechnology, medicine and agriculture have been described or isolated and these await evaluation.

There has been a growing prejudice against taxonomy in recent times. Yet it remains one of the most difficult among scientific disciplines, requiring keen powers of observation, thorough understanding of variation and its significance and the ability to form judgements concerning the delimitation of various taxa in any scheme of classification. Scientists like Subramanian who combine these qualities are indeed becoming rare and unless taxonomy is given its due place we would continue to be ignorant about our enormous wealth of mycobiota. The constant search for newer organisms for use in microbial processes has to be aided by scientists who have special taxonomic knowledge.

Subramanian has chaired special sessions in International Conferences too numerous to be listed here and he was also leader of the delegations which represented India at the two International Mycological Congresses so far held. The most prestigious of his international assignments is his election as President of the International Mycological Association in 1977 and the pivotal role he played in that capacity in securing a key position for Mycology in the International Union of Biological Sciences. An aspect that singles out Subramanian from other specialists is his commitment to and support for science as a whole. He has found time to write an exciting book THE MICROBES for school children (1974, NCERT, New Delhi). He is editor-in-chief of KAVAKA (Transactions of the Mycological Society of India).

Many honours have been bestowed upon Subramanian, of which mention may be made of the Shanti Swarup Bhatnagar Prize (1965), Birbal Sahni Medal (1972), Rafi Ahmed Kidwai Prize (1972–73) and the Jawaharlal Nehru Fellowship (1976–78). He was Vice-President, XIII International Botanical Congress, Sydney, 1981 and a Member of the University Grants Commission, 1979–82. The Indian National Science Academy elected him a Fellow in 1960. He is a Corresponding Member of the Belgian Royal Academy of Foreign Sciences (1978) and of the Mycological Society of America (1982).

Subramanian's secret of youthfulness springs from abundant curiosity, perennial enthusiasm to learn, subtle humour, passion for classical music, and above all deep interest in philosophy. We wish him many years of activity, good health, happiness and the much needed time to complete the stock of unfinished tasks. To his wife Lakshmi and his two sons who have stood by him in his work we offer greetings and best wishes.

The spontaneous response of several authors from India and abroad to our invitation to contribute papers for this special issue is gratefully acknowledged.

New Delhi
20 February 1985

H. Y. Mohan Ram
Member, Editorial Board
Proceedings, Plant Sciences

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 85–90.
© Printed in India.

Effect of soil texture on microbial abbreviation of saprophytic survival by the take-all fungus of wheat

S D GARRETT
University of Cambridge Botany School, Downing Street, Cambridge CB2 3EA, England

Abstract. Median periods of saprophytic survival by *Gaeumannomyces graminis* var. *tritici* in colonized wheat straws are shorter in clay-loams than in soils of lighter texture. This reduction has been ascribed to the microflora of clay-loam soils, and especially to competition by commensal bacteria for the sugars released by fungal cellulolysis of the wheat-straw tissue. Model experiments on the growth of colonies of four cereal foot-rot fungi on filter-paper in the presence of bacteria showed that the slowly growing colonies of *G. graminis* var. *tritici* caused a loss in paper dry weight that was nearly five times as great, per unit area, as that by colonies of *Fusarium culmorum*, which grow four times as fast in the presence of bacteria. By their rapid uptake of sugars, commensal bacteria prevent the regulation of cellulase enzyme induction by catabolite repression, so that cellulolysis continues at a maximum rate. So this bacterial competition for sugars hastens exhaustion of the substrate and thus shortens survival of the take-all fungus.

Keywords. *Gaeumannomyces graminis* var. *tritici*; saprophytic survival.

1. Introduction

Anniversaries give much pleasure to those taking part in them and so I welcomed the invitation to write an article for the special issue of these Proceedings that has been arranged to celebrate the 60th birthday of Prof. C V Subramanian. He has been a good friend of mine for more than 30 years, ever since he came to work in our sub-department of Mycology in the Cambridge Botany School. Since then he has been a pioneer in his voyages of discovery among fungi, and has established more than fifty new genera. The publication of his great monograph of the Hyphomycetes in 1971 was a notable event for fungal taxonomists all over the world. But his interests in taxonomy led Prof. Subramanian into a wider interest in fungal ecology, which he has equally enriched by his flair for discovery. This is well shown by his recent book, *Hyphomycetes: Taxonomy and Biology*, published by Academic Press in 1983; an extraordinary volume and diversity of information has been conveyed in so readable a style that it is easy to assimilate. Prof. Subramanian's international standing was recognized by his term as President of the International Mycological Association; his social gifts proved invaluable in this office, in which he was a felicitous ambassador for his country as well as for his science.

The opportunity for reflection afforded me by the invitation to write this paper has enabled me at last to understand some observations that I had published 45 years ago. This understanding has been enlarged by the results of some experiments made over the last few years, in a way that I had not foreseen. Such a bringing together of two sets of data, not hitherto associated, is sometimes a source of new ideas.

2. Saprophytic survival by the take-all fungus

After harvest of a crop affected by take-all the fungal pathogen, *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker, continues to survive as a saprophyte in the dead, infected tiller-bases of wheat or barley after the stubble has been turned under the soil. Microscopical examination of colonized wheat straw showed me that mycelium of *G. graminis* continued to develop within the tissues after the straw had been buried in soil; this development was greater, and continued for a longer period when there was a sufficient supply of nitrate-nitrogen diffusing into the straw tissue from the surrounding soil (Garrett 1940). For maximum longevity of survival, *G. graminis* needs a supply of nitrogen at the rate of *ca* 0·5 g N for 100 g air-dry wheat straw. This permits a continued though slow development of hyphal branches on the mycelium and these continue to decompose cellulose and other insoluble carbon substrates in the straw tissue.

Naturally infected straw provided only a small fraction of the volume required, and so most experiments were made with artificially colonized units, which were cut from fresh long straw (3·75 × 0·4 cm), each unit including a node at the lower end. These straw units were autoclaved moist in flasks, inoculated with *G. graminis* and incubated for 1 month at 25°C. Fifty such colonized straws were buried in glass jars holding 200 ml soil; two jars were taken from each treatment-series at successive sampling times for estimation of the percentage of straws in which *G. graminis* was still viable; this was done by means of a wheat-seedling test for infectivity (Garrett 1938, 1940).

The original tables of data on survival gave the percentage of straws in which *G. graminis* was still viable at successive sampling times, at intervals of several weeks. Such downwards trends in percent survival cannot quickly be compared and so for a review of available data (Garrett 1976) I introduced the median survival period (S50); this is the period for which the fungus has survived in 50 % of the straws. S50 periods can be approximately determined by inspection of the data but a more accurate method is to calculate the regression of percent survival (y) on time (x). Then by giving y a value of 50 in the regression equation, the time (x) to 50% survival can be calculated. It is best to calculate the regression equation from that time after which a significant and continued decline in viability of *G. graminis* has begun; the survival points then fall quite closely along a descending straight line, for which alone calculation of a regression equation is permissible. The S50 period thus determined should then be compared with the original data to see that no mistake has been made; this I failed to do in an earlier published version of table 1 in the next section; I have now corrected my original errors.

3. Effect of soil texture on survival

In an early experiment (table 3, Garrett 1938) I compared survival by *G. graminis* in eight types of soil. Two soils stood out from the other six, because S50 periods in them were less than half. Both of these soils were clay-loams; one (pH 6·8) was from an arable field on the Rothamsted Experimental Station and the other (pH 8·2) from over the chalk at Whitchurch in Hampshire. At that time it was not possible to conclude that the short S50 periods in these two soils were due to their texture; I had not determined their nitrogen status. But in a later and more comprehensive experiment (table 5,

Garrett 1940) I was able to eliminate soil nitrogen supply as a complicating facto because *G. graminis* survived for longer in the soil with lower nitrogen content; this wa a light-textured sandy soil (pH 7·2) from Bridgham in Norfolk; its total nitroge content was 0·12 %. The second was a clay-loam (pH 6·8) from a Rothamsted arabl field, with a total nitrogen content of 0·20 %.

There were three treatment-series set up for each soil (i) straws colonized b *G. graminis* in axenic culture and buried in glass jars of soil in the laboratory, which wa maintained at a mean temperature of 18°C (ii) similar colonized straws buried in small field plot of each soil placed in a shallow pit (iii) naturally infected tiller-bases c wheat buried in a field plot. The naturally infected tiller-bases were collected on August 1938 from a single crop of wheat; they were trimmed of roots and cut down to size comparable with that of the colonized straws and then stored cool and moist unt the experiment was set up on 30 August. S50 periods (in months) have been calculate from table 5 in Garrett (1940) and are shown in table 1 which shows that, for colonize straws buried in laboratory containers of soil, the S50 period for Bridgham soil wa 1·75 times that in Rothamsted soil. In similar colonized straws buried in field plots, th S50 period in Bridgham soil was 1·4 times that in Rothamsted soil. The longer S5 periods in field plots that in the laboratory can be ascribed chiefly to the low so temperatures during winter.

Table 1 also shows that the S50 period for naturally infected straws buried in the fiel plot of Rothamsted soil was almost identical with that for colonized straws, thu justifying the use of colonized straws in most survival experiments. But the S50 perio for infected straws buried in the field plot of Bridgham soil was only 0·4 month longe than that for similar infected straws buried in the plot of Rothamsted soil. At the tim that these data were published, I had been unable to explain why the S50 period fo infected straws in the Bridgham field plot had been so much shorter than that fo colonized straws. But I had neglected a difference between colonized and infecte straws that now seems to account for this discrepancy. When colonized straws are fir buried in the soil, they are occupied by a pure culture of *G. graminis* alone. But tille bases of wheat naturally infected by *G. graminis* have already been invaded by oth soil micro-organisms even while the crop is still standing in the field before harvest. Th collection of infected straws used for this experiment had been taken from a clay-loa soil over the chalk in Cambridgeshire, and so the straws were likely to have bee invaded by micro-organisms from that soil before they were collected.

There is good evidence for this supposition. From tiller-bases of wheat and barle thoroughly infected by *G. graminis* and collected from seven widely separated localitie

Table 1. Median survival periods of *Gaeumannomyces graminis* var. *tritici*.

	S50 periods (months)	
	Rothamsted	Bridgham
Colonized straws		
Lab. containers	2·61	4·56
Field plots	7·03	9·92
Naturally infected straws		
Field plots	7·09	7·48

in England, Sadasivan (1939) isolated *Fusarium culmorum* (W. G. Sm.) Sacc. much more often than *G. graminis*, which is a poor competitor on the agar plate. Chambers and Flentje (1969) recorded numbers and species of soil fungi isolated from straws colonized by *G. graminis* and then buried in soil; numbers of fungi thus isolated from the internal tissues were more than doubled if the soil had been enriched with nitrate. Soil bacteria, which usually invest hyphal surfaces, follow soil fungi into the straw tissue, originally colonized by *G. graminis*.

So we can conclude that these naturally infected straws buried in the field plot of Bridgham soil were characterized by an S50 period little longer than that for the Rothamsted soil, because they had already been invaded by the microflora of another clay-loam soil. This suggests that it is the microflora of such clay-loam soils, rather than a direct physical effect of their texture, that shortens survival by *G. graminis*.

4. Discussion

We now have to consider which element of the soil microflora is the most likely to shorten survival of *G. graminis* in clay-loams. By comparison with light-textured soils, clay-loams have a higher moisture-holding capacity, and secondly they offer a large area of internal surfaces, which provide sites for the development of bacteria when a substrate becomes available; the physics of attachment by bacteria to clay particles has been discussed by Marshall (1975). Thirdly, these soils are well buffered against change in pH and have a high base-exchange capacity. All these characteristics make such soils more favourable than soils of lighter texture for growth of bacterial populations whenever soil moisture permits and energy-substrates are offered. Secondly, as every mycologist knows, bacteria are successful competitors against fungi for the soluble nutrients of the agar plate; this is because the high metabolic rate of bacteria, due to the small size of their cells, gives them a considerable advantage over fungal cells in competition for nutrients.

During recent years, my studies on colonization of unsterilized filter-paper by cereal foot-rot fungi have shown that bacteria are powerful competitors for the sugars released by fungal cellulolysis of the paper. Colonization of the paper circles by these cellulolytic fungi requires an adequate inoculum potential of the PD agar inoculum disks (5 mm diam.) at paper moisture contents of both 75 and 100 % saturation with the mineral salts solution. This is why adventitious colonies of other cellulolytic fungi so rarely appear on the papers, because the inoculum potential of their propagules is too low (Garrett 1980). But if streptomycin is added to the minerals solution to control bacteria, then colonies of the inoculated fungi grow faster and numerous colonies of other cellulolytic fungi appear on the papers (Garrett 1983a). The next step was to determine loss in oven-dry weight by single paper circles, each inoculated at four loci with inoculum disks of one of the four fungi, which were *Fusarium culmorum*, *Cochliobolus sativus* (Ito and Kuribay.) Drechsl. ex Dastur, *Curvularia ramosa* (Bain.) Boedijn, and *G. graminis* var. *tritici*. Five paper circles were thus inoculated with each fungus, and then incubated in a water-saturated atmosphere for 3 weeks at 20° C, after which diameters of fungal colonies were measured, and mean dry weight losses by single papers were determined (Garrett 1983b). The figures in table 2 have been taken from the series of unsterilized papers adjusted to a moisture content of 75 % saturation, so that growth rates of the four fungi could be compared with those on sterile paper at a

Table 2. Colonization of unsterilized filter-paper by cereal foot-rot fungi.

Fungi	Growth rate over paper (mm. 24 hr $^{-1}$)		Wt. loss by single paper (mg)	Area of 4 fungal colonies (cm^2)	Wt. loss by 1 cm^2 colony (mg)
	Sterile	Unsterile			
F. culmorum	3·75	0·42	5·9	16·3	0·36
C. sativus	2·38	0·33	7·2	11·3	0·64
C. ramosa	1·68	0·21	9·8	6·0	1·63
G. graminis	1·25	0·10	4·6	2·7	1·73

similar moisture content (Garrett 1984). In this article, I had also recorded weight losses by single circles, but the technique with sterile paper was so different from that adopted for unsterilized paper that comparison between weight losses in the two series was unreliable, though the simpler comparison between growth rates does not need to be thus qualified and so is given in table 2.

The first two columns in table 2 show that the associated bacteria developing on the unsterilized paper reduces fungal growth rate to a percentage varying from 8 for *G. graminis* to 13 for *C. sativus*. Two separate trials made during these experiments on weight losses by unsterilized paper showed that no weight had been lost by papers not inoculated with fungi, so that no cellulolytic bacteria could have been active. So growth of fungal colonies must have been restricted by commensal bacteria, competing with the fungi for sugars released by fungal cellulolysis. We can expect that the two fast-growing fungi would have been less affected by bacterial competition than the two slow-growing ones; on the nutrient agar plate, fast-growing fungi can often grow away from an area colonized by bacteria, whereas slow-growing fungi are usually unable to do so. But the effect of this bacterial competition has not been to reduce fungal cellulolysis rate (table 2, 5th column); on the contrary it has increased loss in weight by 1 cm^2 colonized area, in a progression from *F. culmorum* (0·36 mg) to *G. graminis* (1·73 mg). Colonies of *G. graminis* have been 4·8 times as active in cellulolysis per unit area as those of *F. culmorum*.

This effect of commensal bacteria in increasing fungal cellulolysis rate can be explained by the mechanism that controls production of cellulases and other adaptive enzymes, known as catabolite repression. As soon as the concentration of sugars, released by cellulolysis, reaches a certain critical level, induction of further cellulase enzymes is repressed until sugars fall once more below the critical level. So catabolite repression tends to keep the rate of consumption of insoluble carbon reserves of a cellulosic substrate at an economic level for the fungal colonizer. As Montgomery (1982) has noted, this mechanism of control is likely to be effective for timber-rotting fungi, invading the closely knit tissues of wood. But it is likely to be less effective for fungi exploiting the looser fabric of wheat-straw tissue, because soil bacteria soon follow fungal hyphae into the tissue. By their rapid uptake of sugars, commensal bacteria are likely to keep sugars at a level below that needed for catabolite repression to operate. So fungal cellulolysis continues unchecked, at a rate higher than it would be in the absence of bacterial competition. In their uptake of sugars, bacteria lock up in their cells an equivalent amount of nitrate–nitrogen, which limits saprophytic survival by *G. graminis*. So the net effect of bacterial competition is to hasten exhaustion both of

carbon reserves in the straw tissue and of nitrate from the surrounding soil. This must shorten survival by *G. graminis* and, for reasons given above, the effect may be chiefly responsible for the shorter S50 periods of this fungus in clay-loam soils. This effect of soil bacteria may be one way by which they contribute to biological control of the take-all disease, but it is not the only way. Cook and Baker (1983) have reviewed the evidence for their conclusion that fluorescent pseudomonad bacteria are the agents of "take-all decline", which begins to operate after wheat or barley have been grown consecutively on the same field for 3–4 years. In the early years of my career, many mycologists regarded bacteria chiefly as a nuisance in their culturing of fungi; now we have to think otherwise.

References

Chambers S C and Flentje N T 1969 Relative effects of soil nitrogen and soil organisms on survival of *O. graminis*; *Aust. J. Biol. Sci.* **22** 275–278

Cook R J and Baker K F 1983 *The nature and practice of biological control of plant pathogens* (St. Paul, Minnesota: American Phytopathological Society) p. 256

Garrett S D 1938 Soil conditions and the take-all disease of wheat. III. Decomposition of the resting mycelium of *Ophiobolus graminis* in infected wheat stubble buried in the soil; *Ann. Appl. Biol.* **25** 742–766

Garrett S D 1940 Soil conditions and the take-all disease of wheat. V. Further experiments on the survival of *Ophiobolus graminis* in infected wheat stubble buried in the soil; *Ann. Appl. Biol.* **27** 199–204

Garrett S D 1976 Influence of nitrogen on cellulolysis rate and saprophytic survival in soil of some cereal foot-rot fungi; *Soil Biol. Biochem.* **8** 229–234

Garrett S D 1980 Colonization of unsterilized filter-paper by cereal foot-rot fungi; *Trans. Br. Mycol. Soc.* **74** 259–263

Garrett S D 1983a Factors limiting colonization of unsterilized filter-paper by cereal root-infecting fungi; *Soil Biol. Biochem.* **15** 101–103

Garrett S D 1983b Weight losses of unsterilized filter-paper caused by colonies of cereal foot-rot fungi; *Trans. Br. Mycol. Soc.* **81** 421–423

Garrett S D 1984 Factors controlling growth rate of cellulolytic fungi on sterile filter-paper; *Proc. Indian Acad. Sci.* (*Plant Sci.*) **93** 189–194

Marshall K C 1975 Clay mineralogy in relation to survival of soil bacteria; *Ann. Rev. Phytopathol.* **13** 357–373

Montgomery R A P 1982 The role of polysaccharidase enzymes in the decay of wood by basidiomycetes; in *Decomposer basidiomycetes* (eds) J C Frankland and J N Hedger (Cambridge: University Press) pp. 51–65

Sadasivan T S 1939 Succession of fungi decomposing wheat straw in different soils, with special reference to *Fusarium culmorum*; *Ann. Appl. Biol.* **26** 497–508

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 91–97.
© Printed in India.

Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia

K SIVASITHAMPARAM and I ROWLAND*

Soil Science and Plant Nutrition Group, University of W.A., Nedlands, 6009, Western Australia

* Western Australian Department of Agriculture, South Perth, 6151, Western Australia

Abstract. Soils were sampled for over two years from two blocks with differing rotational history in a paddock at Newdegate, Western Australia to assay for propagule numbers of the take-all fungus and disease severity. Grasses were found to be capable of carrying the pathogen effectively and were almost as hazardous as the cereal crop in the perpetuation of the inoculum. The effects of time on the most probable number (MPN) and the disease severity assay (DSA) were mostly similar. There appears to be a drop in both these measurements during certain periods, commonly in August/September and in May/June. There is also an unexpected increase in MPN and DSA during the summer months.

Keywords. Take-all fungus; inoculum; cereal root rots; pasture.

1. Introduction

Gaeumannomyces graminis (Sacc.) Arx and Olivier var *tritici* Walker (= take-all fungus) survives in soil as mycelia in residues of hosts (Hornby 1981). Work done at Rothamsted, UK since the late sixties has thrown some light into the behaviour of the propagules in the soil in the presence and absence of hosts (Hornby 1975, 1981).

Under Australian conditions the pathogen survives through a harsh period of dry heat of summer. The residues in the temperate wheat growing countries of the Northern Hemisphere however, are subjected to a wet cold winter following harvest of the crop. The differences in cropping practices (Perry 1981), soil forms (McGarity 1975) and rainfall patterns (Nix 1975) associated with Australian wheat farming would make it impractical to extrapolate data from UK to the Australian situation. This paper reports the behaviour of inoculum of the take-all fungus at a field site in the wheat belt of Western Australia. The aim of the study was to follow the status of the inoculum in a paddock through time.

2. Materials and methods

2.1 *Sampling site*

The blocks chosen were from a long-term rotational trial (67 N 4) at Newdegate, Western Australia, (latitude 33°07, longitude 118°50). The soil is a grey sand over gravel at 20–30 cm (Northcote *et al* 1975; Dy 5·41) and the site had a history of take-all. Samplings were made from three plots (10 m^2 each) selected at random within two blocks of differing rotational history. The cropping history of the blocks are set out in table 1.

The land was cleared in 1952. Experiments on rates of superphosphate on wheat

Table 1. History of two blocks selected from a rotation trial at Newdegate, Western Australia.

	'67	'68	'69	'70	'71	'72	'73	'74	'75	'76	'77	'78	'79	'80	'81
Block 13	P	P	P	C	C	P	P	P	P	C	C	P	P	P	P
Block 23	P	P	P	C	P	P	P	P	C	P	P	P	P	C	P

P = Pasture; C = Wheat

were conducted at the site from 1953–1958. In 1959 it grew oats, the stubble was sown with subterranean clover (cv. 'Dwalganup') in 1960. Amendments with zinc (1953) copper (1954), zinc (1959) and copper and zinc (1961) were also made. From the start of the trial (1967) both blocks received annual dressings of 112 kg/ha superphosphate—topdressed on pasture or drilled with seed for the crop. Muriate of potash (KCl) 100 kg/ha was topdressed in 1972 on both blocks.

2.2 *Details of the blocks with history*

1977: Block 13—(2nd year crop). 21/4 stubble burnt—fire harrowed, 12/5 light cultivation with combine, 13/5 light harrowing, 25/5 scarified and harrowed, 31/5 sown—with winter wheat (cv. 'Gamenya'), 19/10 dry matter yield of tops at anthesis—1725 kg/ha, 16/12 grain yield—193 kg/ha. Serious take-all and *Septoria* infections. Heavily infested with capeweed (*Arctotheca calendula* (L.) Druce) and annual ryegrass (*Lolium rigidum* Gaud.). Block 23—(2nd year pasture), observations on 20/10: clover 50%, grass 50% (60% barley grass (*Hordeum leporinum* Link) and 40% brome grass (*Bromus driandrus* Roth).

1978: Block 13—(1st year pasture after 2 crops), pasture cut on 17/10—total dry matter 2417 kg/ha; clover 0%, broadleaf weeds 65%, grass 35% (self sown wheat and ryegrass). Block 23 (3rd year pasture), pasture cut on 17/10—total dry matter 3050 kg/ha; clover 80%, broadleaf weeds 10%, grass 10%.

1979: Block 13—(2nd year pasture), observations on 15/8: Poor clover stand (10% of cover). Grass dominated by ryegrass. Block 23 (4th year pasture), poor clover, although 20% of cover, only made up of small plants. Erodium (*Erodium botrys* (Cav.) Bertol) dominant, a few grasses.

1980: Block 13—(3rd year pasture), Resown with subterranean clover (cv. 'Nungarin') at 18 kg/ha on 30/4. Block 23 (1st year crop), 22/4 Dicamba (@) 1·2 L/ha—for sorrel (*Rumex acetosella* L.) control, 7/5 scarified, 3/6 worked back with combine, 12/6 sown—with wheat (cv. 'Gamenya'), 30/9 dry matter yield of tops at anthesis; 2596 kg/ha, 1/12 grain yield, 1083 kg/ha. Dry season affected crop, with the months of July, August and September being particularly dry.

Soil analysis: Soil for analysis was recovered from 0–10 cm, air dried and passed through a 2 mm seive. The soil was sampled in each year shown during the period (March-May) prior to cultivation (see table 2). The pH of these soils was measured in March 1978 in a 1:5 suspension of soil and water and was 5·5 and 5·4 for Blocks 13 and 23 respectively. The soils were analysed for easily reducible carbon (by the Walkley-Black method) and for total nitrogen (by the Kjeldahl method). Results from the two blocks are shown in table 2.

Table 2. Percentage carbon and nitrogen in soil sampled at the beginning of each year*.

	1977		1978		1979		1980	
	Block 13	Block 23	Block 13	Block 23	Block 13	Block 23	Block 13	Block 23
% C	0·70	0·81	0·61	0·74	0·71	0·95	0·72	0·87
% N	0·050	0·060	0·040	0·051	0·046	0·066	0·049	0·066

* Soil from top 10 cm, passed through a 2 mm sieve before analysis.

Table 3. Rainfall (*R*) in mm and maximum air temperature (*T*) °C for the period of study.

	Jan		Feb		Mar		Apr		May		Jun		Jul		Aug		Sept		Oct		Nov		Dec	
	R	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*	*R*	*T*
1977	1	32	12	32	0	28	5	25	51	18	33	17	33	17	58	17	63	19	54	23	5	26	12	33
1978	34	32	64	31	0	30	18	26	29	20	76	16	89	15	29	17	37	19	13	23	9	27	6	29
1979	15	—	37	—	20	—	8	—	25	—	68	—	40	—	55	—	17	19	6	24	13	27	8	31
1980	9	33	46	29	1	30	25	23	22	21	43	16	24	16	31	17	15	21	34	21	3	28	67	30
1981	11	30	32	27	6	28	9	26	61	19	48	14	51	15	43	16	18	21	5	24	36	23	1	28

– denotes no recording taken.

2.3 *Sampling method*

Samplings were done at random points within a grid made of nylon ropes. The grid was made by connecting the sides of the outer perimeter (10 × 10 m) of the main rope frame with thinner ropes at metre intervals so that when the whole rope-grid was spread out there were 100 × 1 m^2 areas available for sampling. At each sampling time soil slabs 20 cm × 20 cm and about 5 cm deep were lifted with a spade (just as one does with turf slabs). A composite of three such slabs collected at random (weighing around 3 kg) formed one sample representing each grid. There were 3 such grids in each of the 2 rotation blocks.

2.4 *Inoculum studies*

Soil samples were air dried and sieved through a 5 mm sieve. The most probable number (MPN) technique (Maloy and Alexander 1958) was employed to estimate the numbers of infective propagules in the sieved fraction. The soil was diluted by ten fold steps to 10^{-5} with subsoil yellow sand and potted in 5 × 250 g lots for each dilution. The pots were seeded (5 per pot) with wheat (cv. 'Gamenya') and were maintained in phytotrons at 15 ± 1°C. The roots of these plants were washed free of soil 6 weeks after seeding, floated in a white enamel dish of water and scored under low magnification for the presence or absence of take-all lesions.

2.5 *Disease severity assay*

The disease scoring for roots of wheat grown in undiluted field soil was considered to be the 'disease severity assay' of the soil. Root rotting was scored on a 0–5 scale with

steps at 0·5 intervals. Plants with a 0 score showed no infection and those with all roots rotted received a score of 5.

3. Results and discussion

Although the numbers of propagules (MPN) and the infectivity of the soil as shown by the DSA varied with time within both blocks there was a parallel effect of time on both these measurements (figures 1 and 2). The drop in DSA and MPN was noticeable around spring (August/September) for 1978 and 1979 in both blocks, the drop in 1979 in Block 13 being less marked. Similar decreases for MPN were also observed in certain May/June periods. There is also an increase of MPN and DSA during certain months of winter and surprisingly during dry summer months as well. Replacement of the cereal crop with pasture species does not appear to cause any great reduction in the infectivity of the soils.

The MPN and to a lesser extent the DSA appear to behave in a similar pattern in both blocks. In 1977, Block 13 carried a wheat crop which was severely affected by take-all. Although no take-all assay was made for Block 23 during this period, records show that it carried a 50% cover of grasses made up of barley (60%) and brome (40%) grasses both of which are known to be susceptible to take-all (Nilsson and Drew-Smith 1981). This dominance of grasses may be responsible for the high level of infectivity of Block 23 soil in 1978. The authors are unable to extend any explanation of the drop in MPN and DSA in May-June or August-September. Increase in the number of propagules and DSA during winter months in the absence of crops is attributed to susceptible

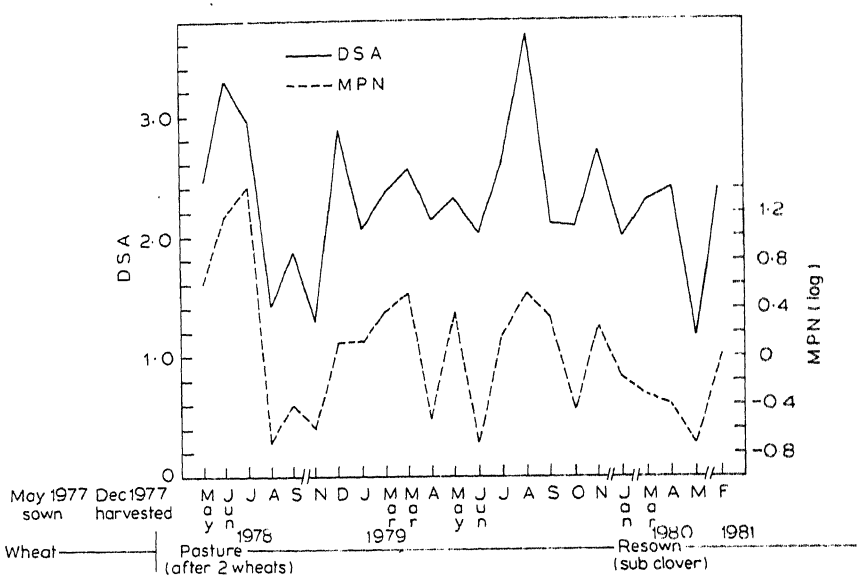


Figure 1. Disease severity assay (DSA) and most probable number (MPN) of propagules of the take-all fungus in soils sampled at different times of the year in Block 13 within a paddock at Newdegate, Western Australia. *N.B.* Samplings were done at fortnightly intervals in March 1979. Table 4 below is presented to show relative differences among either DSA or MPN values in figure 1.

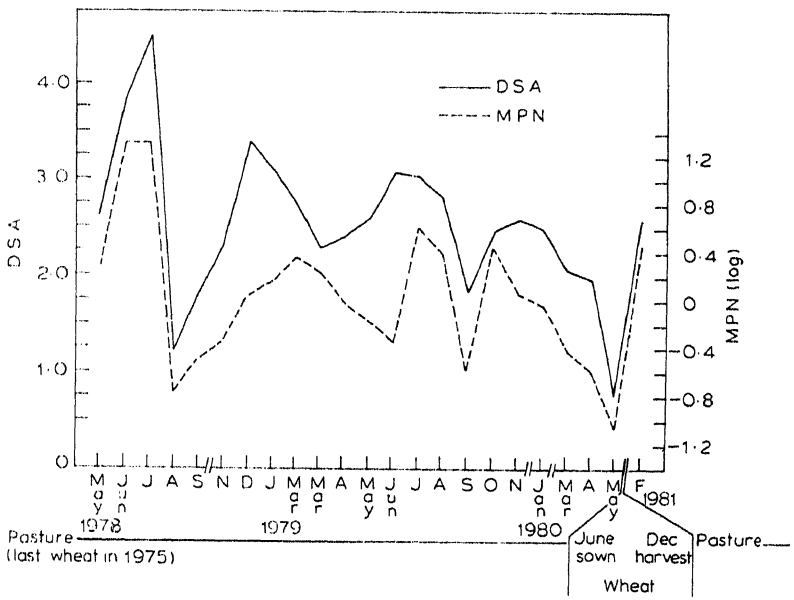


Figure 2. DSA and MPN of propagules of the take-all fungus in soils sampled at different times of the year in Block 23 within a paddock at Newdegate, Western Australia *N.B.* Samplings were done at fortnightly intervals in March 1979. Table 5 below is presented to show relative differences among either DSA or MPN values in figure 2.

grasses. The peaks during summer (around December) in both blocks are very interesting as soil moisture levels at this time of the year are unlikely to be sufficient to promote saprophytic growth of the fungus in the absence of living host. Such growth is unlikely at our summer temperatures, even if the moisture was present, owing to the inhibitory effect of temperature (Grose *et al* 1984). Post-harvest saprophytic growth in residues has been suggested to explain increase in inoculum levels following harvest in the UK (Hornby 1975), where soil moisture and temperature conditions would not be too harsh for such growth.

The processes of air-drying and sieving could encourage fractionation of the dry and sometimes brittle inoculum. Although this experimental artifact may exist in our investigation it does not explain why the increase in summer months of the MPN is also accompanied by increases in DSA. Air-drying and sieving of the soil was employed to bring about a uniformity in the weighing and dilution of the soil which unlike soils in UK goes through distinct periods of wetness and dryness. Sieving of the soil is presumed to remove residues such as the large crowns etc. which could be significant as natural inoculum. Making ten-fold dilutions with a soil harbouring such large residues and gravel stones was found to be impracticable. Both the DSA and MPN are therefore those of the sieved fractions which are considered to be sufficiently indicative of their potency as the soil-borne inoculum. It is noteworthy that pasture species are capable of sustaining populations of the pathogen and are almost as hazardous as the cereal crop in maintaining infectivity of the soil as shown by the DSA values. This is in contrast to the grassy pastures in UK which often have a suppressive effect on the disease (Deacon 1981). It is likely that the differences in soil environment and cropping practices

Table 4. Relative differences among either DSA or MPN values in figures 1 and 2 respectively. Values assigned with same letters are not significantly different from each other (Duncan's Multiple Range Test P = 0·05). Comparisons may be made within DSA or MPN values but not between them.

	1978							1979											1980				1981
	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Jan	Mar	Apr	May	Feb
DSA	FG	I	H	A	B	A	H	BCD	DEF	FG	BCDE	CDEF	BC	FG	J	BCDE	BCD	GH	B	CDEF	EF	A	EF
MPN	BCD	E	F	A	AB	A	ABCD	ABC	ABCD	A	D	A	ABCD	ABCD	ABCD	ABCD	A	CD	AB	AB	A	A	ABCD

Table 5. Relative differences among either DSA or MPN values in figures 1 and 2 respectively. Values assigned with same letters are not significantly different from each other (Duncan's Multiple Range Test P = 0·05). Comparisons may be made within DSA or MPN values but not between them.

	1978							1979											1980				1981
	May	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Mar	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Jan	Mar	Apr	May	Feb
DSA	EFG	K	L	B	C	DE	J	HIJ	FGH	DE	DEF	EFG	IJ	HI	GHI	C	DEFG	EFG	DEFG	CD	C	A	EFG
MPN	AB	E	E	A	AB	AB	C	C	DE	DE	C	ABC	AB	DE	DE	A	DE	C	C	AB	AB	A	DE

between UK and Western Australia may explain the lack of the cleaning effect that a pasture phase has in the UK. The dominance of *Phialophora graminicola* in pastures in UK has been considered (Deacon 1981) to be the cause of reduction in take-all of wheat following such pastures. Similar activity of *P. graminicola* is not evident in grassy pastures under Western Australian conditions (J S Yeates pers comm).

The results of this investigation indicate that field-inoculum behaviour at a site in the Western Australian Wheatbelt is different from that of the same pathogen observed in wheatfields in the UK. This is to be expected considering the mediterranean climate (table 3) and the soil types of the Western Australian Wheatbelt. It is also noteworthy that the soils are acidic contrasting with the alkaline take-all prone soils in the UK (Garrett 1981). Isolates of the pathogen from the Western Australian Wheatbelt have been recorded to have pH optima for growth in the acidic range (Sivasithamparam and Parker 1981). Detailed work into the inoculum and its relationship to the disease need to be done to understand the field biology of the fungus under Australian conditions.

Acknowledgements

This research was supported by a grant from the Western Australian Wheat Industry Committee. The authors are grateful to Drs D Hornby and G C MacNish and Mr P Cotterill for valuable discussions and suggestions. They thank Mr L Smith for technical assistance.

References

Deacon J W 1981 Ecological relationships with other fungi: competitors and hyperparasites; in *Biology and control of take-all* (eds) M J C Asher and P J Shipton (London: Academic Press) 75–101

Garrett S D 1981 *Biology of root-infecting fungi* (Cambridge: University Press)

Grose M J, Parker C A and Sivasithamparam K 1984 Growth of *Gaeumannomyces graminis* var *tritici* in soil: effects of temperature and water potential; *Soil Biol. Biochem.* **16** 211–216

Hornby D 1975 Inoculum of the take-all fungus: nature, measurement, distribution and survival; *EPPO (Eur. Mediterr. Plant Prot. Organ.)* **5** 319–333

Hornby D 1981 Inoculum; in: *Biology and control of take-all* (eds) M J C Asher and P J Shipton (London: Academic Press) 271–293

McGarity J W 1975 Soils of the Australian wheat growing areas; in: *Australian field crops*, Vol. 1: Wheat and other temperate cereals. (eds) A Lazenby and E M Matheson (Sydney: Angus and Robertson) 227–25

Maloy O C and Alexander M 1958 The "most probable number" method for estimating populations of plant pathogenic organisms in the soil; *Phytopathology* **48** 126–128

Nilsson H E and Drew-Smith J 1981 Take-all of grasses; in: *Biology and control of take-all* (eds) M J C Asher and P J Shipton (London: Academic Press) 433–448

Nix H A 1975 The Australian climate and its effect on grain yield and quality; in: *Australian field crops* Wheat and other temperate cereals (eds) A Lazenby and E M Matheson (Sydney: Angus and Robertson) Vol. 1 pp. 183–226

Northcote K H, Hubble G D, Isbell R F, Thompson C H and Bettenay E 1975 *A description of Australian soils* (Melbourne: CSIRO)

Perry M W 1981 United Kingdom Wheat Industry on the move; *J. Agric. W. A.* **22** 139–143

Sivasithamparam K and Parker C A 1981 Physiology and nutrition in culture; in: *Biology and control of take-all* (eds) M J C Asher and P J Shipton (London: Academic Press) 125–150

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 99–109.
© Printed in India.

Specificity and penetration of tissues by mycorrhizal fungi

J L HARLEY
The Orchard, 20 Oxford Road, Old Marston, Oxford OX3 0PQ

Abstract. Although mycorrhizas are diverse in structure they fall into two physiological groups. Those in which the host provides carbon compounds and those in which the fungus does so. The fungi may belong to any of the great taxa, but those of each taxon do not necessarily form one kind of mycorrhiza or consort with particular taxa of hosts. The species of fungi are not specific to any species of host, and although genera of fungi may be restricted to families or genera of host, most are of very wide host range. Some species or strains of fungi can form mycorrhizas of different structure with different hosts and some hosts may form different kinds of mycorrhiza with different fungi.

Those fungi which consort with hosts which provide carbon compounds have a very limited ability to degrade or use complex carbon polymers. Those that provide the carbon for their symbioses are active in such breakdown. Both kinds may penetrate between or into the cells of the hosts without causing death of cells. This problem is discussed and one hypothesis suggesting a means of penetration by the former, and the prevention of tissue destruction by the latter is put forward.

Keywords. Mycorrhiza; specificity; fungal penetration; symbiosis.

1. Introduction

This essay discusses two fundamental and general aspects of the biology of mycorrhizal fungi, namely their specificity to, and their mode of penetration of, their hosts. It will be seen that these mutualistic symbiotic fungi behave differently, in both these aspects of behaviour, from biotrophic and necrotrophic parasites. These subjects have been discussed in detail with Dr S E Smith during our work together (Harley and Smith 1983) and much of the good in what follows is due to her advice. There are several kinds of mycorrhiza, but they can be grouped under two heads on the basis of their carbon physiology. On the one hand, are those in which the supply of carbon compounds for both host and fungus comes from the photosynthetic activity of the host. On the other, are those mycorrhizal associations where the fungus exports not only inorganic nutrients, but also carbon compounds to the host. In these latter, the host has no power, or a limited power, of photosynthesis during part or the whole of its vegetative life when symbiosis is essential.

In some quarters, it is argued that these two kinds of symbiosis are physiologically so different that they should not be both called mycorrhiza. The latter are sometimes regarded as cases of antagonistic symbiosis or parasitism of the host plant on the fungus. It is not the aim of this essay to argue this matter, but to give a short comparative discussion of a few aspects of the fungal activities in the whole range of phenomena usually comprehended under the name mycorrhiza.

Cutting across the physiological similarities or differences is the structural diversity of mycorrhizas, in which again two extreme kinds can be perceived. On the one hand, are those in which the fungus penetrates between and within the living cells of the infected organ. These are called endomycorrhizas. On the other hand, are those in

which the fungus forms a surface layer, or fungal sheath, on the surface of the infected root and penetrates only between the living cells of its outer layers forming a complex hyphal network, the Hartig net. In this type, called ectomycorrhiza, penetration by the hyphae into the cells only occurs when the organ becomes senescent.

There are several structurally distinct kinds of mycorrhiza which possess characteristics intermediate between these two types of mycorrhiza. They are endomycorrhizal in the penetration of their fungi into the lumina of metabolically active host cells, yet they are like ectomycorrhizas in the formation of an external fungal sheath or Hartig net or both. These are called ectendomycorrhizas, and there are several distinct kinds of them.

These points are emphasised in table 1 and no further discussion of the diversity of types of mycorrhiza will be made here, but the diversity of mycorrhizal fungi will first be considered.

2. Diversity of mycorrhizal fungi

The fungi of mycorrhizal symbioses are taxonomically very diverse for they may belong to any of the great groups of Eumycota; Zygomycotina, Ascomycotina, Basidiomycotina, or Deuteromycotina. Hence one is led to ask whether each structurally different kind of mycorrhiza is associated with a taxonomically distinct group of fungi. Except for the vesicular-arbuscular mycorrhizas which are associated only with Endogonaceae, this is not true (see table 2). It must be stressed that investigation of the fungi of ectomycorrhiza has been the most extensive, and here the widest taxonomic spread is found. Species of at least 64 genera, distributed amongst all the groups mentioned have, so far, been found to be ectomycorrhizal. In ericoid mycorrhiza, many sterile species or strains of fungus had been isolated and proved to be mycorrhizal over the years, and only recently one isolate, *Pezizella ericae*, was identified (Pearson and Read 1973; Read 1974) by its production of fruit bodies in culture. It is also likely that a species of *Clavaria* may be an ericoid endophyte. In most of the other kinds of mycorrhiza, except that in the Orchidaceae, a detailed study of endophytes is yet to be made. The endophytes of the Orchidaceae have received much attention from the early years of the century till recently when Warcup and his associates (Warcup 1971, 1973, 1975, 1981; Warcup and Talbot 1966, 1967, 1970) amongst others have further indicated, by discovering perfect stages, that a wide range of Hymenomycetales of diverse families take part in mycorrhizal infection of orchids.

Since, in a broad sense, none of the larger taxonomic categories of fungi is uniquely associated with a single kind of mycorrhiza, it is pertinent to ask whether a single species of mycorrhizal fungus is always uniquely associated with one kind of mycorrhiza and no other. The answer is definitely in the negative as a few examples will show.

For example, many of the species of Hymenomycete and Gasteromycete which form the ectendomycorrhiza of the arbutoid type (table 2) have been shown to form ectomycorrhizas with members of the Pinaceae (Zak 1973, 1974, 1976a, b; Molina and Trappe 1982). It is important to realize that this shows that the difference in behaviour between these fungi in ectomycorrhizas in which no penetration into the cells occurs and in ectendomycorrhiza where it does, seems to be dependent upon some properties of the host. The same applies to the 'E-type' fungi of ectendomycorrhiza of *Pinus*. These

Table 1. Characteristics of important kinds of mycorrhiza (modified after Harley and Smith 1983)

	Vesicular-arbuscular	Ectomycorrhiza	Ectendo-mycorrhiza of conifers	Arbutoid	Ericoid	Orchid	Monotropoid
Hyphae septate	−	+	+	+	+	+	+
aseptate	+	+	−	−	−	−	−
Hyphae enter cells	+	−	+	+	+	+	+
Fungal sheath formed	−	+	+ or −	+	−	−	+
Hartig net formed	−	+	+	+	−	−	+
Hyphal coils in cells	+	−	+	+	+	+	−
Haustoria dichotomous	+	−	−	−	−	−	−
not dichotomous	−	−	−	−	−	+ or −	+
Vesicles in cells or tissues	+ (or −)	−	−	−	−	−	−
Achlorophylly	− (or +)	−	−	− (or +)	−	+	+
Host Taxa	Bryophytes Pteridophytes Gymnosperms Angiosperms	Gymnosperms Angiosperms	Gymnosperms Angiosperms	Angiosperms (Ericales)	Angiosperms (Ericales)	Angiosperms (Orchidaceae mainly)	Angiosperms (Montotropaceae)

Table 2. Numbers of genera of fungi of different taxa associated with different kinds mycorrhiza.

Fungal Taxon	Vesicular-Arbuscular	Ectomyco-rrhiza	Ectendomycorrhiza		Ericoid	Orchid	Mono-tropoic
			of Conifers	Arbutoid			
Zygo							
Endogonaceae	5	1	(1?)	–	–	–	–
Asco							
Pezizales	–	14	–	–	1	–	–
Elaphomycetales	–	1	–	–	–	–	–
Helotiales	–	2	–	–	–	–	–
Basidio							
Hymenomycetales	–	35		12	1(?)	15	1
Gasteromycetales	–	10		2		–	–
Deutero							
Hyphomycetales	–	2	2	–	α	2	–
"E-type"	–	α	α	–	–	–	–

The numbers are likely to be underestimates. α signifies several strains with different appearance.

fungi, of uncertain taxonomic affinity, form ectendomycorrhizas with *Pinus* ar sometimes *Larix*, yet with *Picea* most form ectomycorrhizas only. (Laiho and Miko 1964; Mikola 1965; Laiho 1965).

A particularly instructive example is found in the fungal endophyte of *Monotrop* isolated by Björkman (1960). This, believed to be a species of *Boletus*, produced typic ectomycorrhiza with *Picea* or *Pinus* but monotropoid mycorrhiza with the a hlorophyllous *Monotropa* with which it was also associated in a three member symbiosis.

3. Specificity of the fungi

In writings on mycorrhiza and elsewhere it is customary to consider degrees specificity (Harley and Smith 1983; Linskens and Heslop-Harrison 1984; Harley 198 whereas writing about the lichen symbiosis Galun and Bubrick (1984) use "specificit to mean "cell to cell interactions in which absolute exclusivity is expressed. Symbiot partners associate only with one another and no other combinations between them a possible". This clearly makes consideration no easier because of the difficulty defining "symbiotic partners". These might be species or subspecific taxa, or uniq genotypes as in gene for gene specificity. The use of "specificity" in this essay therefo relates to "selectivity" in lichen symbiosis.

The previous discussion of mycorrhiza indicates that the species of fungi concern are not restricted to any given species of host. This is true of quite the majority mycorrhizal fungi (see Harley and Smith 1983). There is as yet no case described whe a given species of fungus has been proved to form mycorrhiza with a single species

host plant only. There appear to be genus specific fungi, such as *Alpova diplophloeus* found by Molina (1979) to be restricted to *Alnus*, or *Sebacina vermifera* which Warcup (1981) suggested might be specific to *Caledenia*. More common perhaps are fungi of somewhat wider specificity, as the genus *Rhizopogon*, the species of which are restricted to Pinaceae. On the other hand a very large number of fungal species have a very wide host range. Endomycorrhizal species of *Glomus* e.g. *G. mosseae*, ectomycorrhizal fungi like *Paxillus involutus*, *Cencoccum geophilum*, *Pisolithus tinctorius*, the ericoid fungus *Pezizella ericae*, to name a few, fall into this category. Nor are the species of fungi separable into strains of closer or total specificity. In the examples given for instance, one and the same strain is compatible with many species of hosts.

A complexity arises because many of the host-fungus combinations shown to be compatible in culture, have rarely or never been found in nature. The reasons for this may be many. The cultural conditions may be extremely artificial, e.g. when significant amounts of carbohydrates are available to the fungus in the culture medium. The association may be unstable, in the sense that in the presence of the competing fungi it may be superceded. Lastly perhaps, the search for such a combination in natural sites has not been adequate, for different combinations are difficult to recognize without isolation.

At the moment it does appear that specificity in nature is closer than in culture and this means that habitat conditions are selective. On the other hand, as has been known for a long time, a given individual of a host species may be associated with more than one species of endophyte at one time. For example Zak and Marx (1964) showed this to be true of ectomycorrhiza and Molina *et al* (1978) of vesicular arbuscular mycorrhiza. The latter example is particularly interesting for it was found that in the sites investigated species of *Festuca* associated on an average with three or more species of endophyte in each site.

Another aspect of the low specificity of ectomycorrhizal fungi is in the sequence of development of different kinds of mycorrhiza on a single root system. This was described long ago by Marks and Foster (1967) in plantations of *Pinus radiata*, and has recently been observed during the growth and development of young plants of *Betula* and *Pinus* by Last *et al* (1983) and Mason *et al* (1983). This work implies that, although the root systems at all stages of development are compatible with many species of fungi in culture, there may be selection of particular combinations on plants and roots of different ages or stages of development.

A low degree of specificity of the partners of mycorrhiza or beneficial symbiosis generally is to be expected according to Harley and Smith (1983). There are two reasons for it. There will be a selection against mutations towards resistance in the host plant, and against non-virulence in the fungus because these would tend to break or prevent a beneficial symbiosis. As Vanderplank (1978) emphasized, such selection militates against the development of specificity. There will also be a selection in favour of mutations tending to non-specificity because this increases the probability that compatible hosts and endophytes will meet.

Consider now the relevance of this argument to the two kinds of mycorrhiza mentioned in the first paragraph of this essay—the mycorrhizas of the photosynthesizing green plant, and those of the chlorophyll-free non-photosynthetic host. If the latter were really antagonistic symbioses, *i.e.* parasitism of the higher plant on the fungus, a higher degree of specificity would be expected in the relationship. This does not seem to occur.

4. The penetration of the tissues of the host by mycorrhizal fungi

The fungi associated with the Orchidaceae which have a carbon heterotrophic phase, can absorb carbon readily from the soil or other natural substrate. They can make use of resistant carbohydrate polymers such as cellulose and lignin and also pectins, hemicelluloses etc. (see Burgeff 1936 and Harley 1969). Some, *Armillariella mellea* for instance, are omnivorous parasites of woody and occasionally herbaceous plants, others such as *Rhizoctonia solani* are necrotrophic parasites of herbs, and yet others are wood-destroying saprophytes like *Coriolus versicolor*. All these have the ability in the parasitic or saprophytic mode to break down cell and cell-wall material enzymatically, absorb and translocate soluble products through their hyphae. By contrast the fungi of ectomycorrhizas and ericoid and vesicular-arbuscular mycorrhizas although able to translocate carbon and other nutrient compounds through their hyphae, have very limited or negligible ability to hydrolyse carbohydrate polymers enzymatically. The fungi of arbutoid and monotropoid mycorrhizas, as far as is known, are of the same species as, or similar species to, those of ectomycorrhizas (see Harley and Smith 1983).

It is therefore clear that the penetration of the tissues by mycorrhizal fungi of all kinds is not necessarily associated with the possession of carbohydrase enzymes, nor with their secretion into the surroundings. Where they are formed by mycorrhizal fungi in the saprophytic or parasitic modes, their production or activity must be inhibited in the symbiotic mode. Indeed it has been found that the success or failure of establishment of symbiosis between orchid seedlings and species of *Rhizoctonia* is not related to their parasitic potential with other plants or to their production of carbohydrases (Pérembelon and Hadley 1965; Williamson and Hadley 1970).

The apparent irrelevance of carbohydrase production to the penetration of the cell-walls of the host by mycorrhizal fungi poses a problem. Of course the absence of these enzymes has been judged in many cases by the inability of the fungi to grow in culture with carbon polymers as the sole source of carbon. As Lindeberg and Lindeberg (1977) point out it is still possible that in the apical regions of the hyphae there are active wall-attached enzymes which could aid penetration, and this needs urgent investigation. There are other possible hypotheses which are best discussed in the background of the detailed histological relationship between the symbionts.

5. Position of penetration of the tissues

The penetration of the tissues of the host by the fungus takes place only in those roots (or other organs) which have a primary structure, before the onset of secondary thickening. It occurs behind the apical meristem, for that is never penetrated in active healthy mycorrhizal organs. To be sure, the apex of ectomycorrhizas may early become enclosed in an incipient fungal sheath and remain enclosed as the sheath develops, but the initials and meristem remain free of fungus. Nor does penetration of the tissues take place in regions remote from the apex where the cells are fully formed and mature, with mature cell walls (Atkinson 1975; see Harley and Smith 1983). Similarly, the apex of the roots infected by vesicular-arbuscular mycorrhizal fungi is not colonized. It has sometimes been assumed that it is simply because it is rapidly growing that it escapes infection, but that is unlikely. Regardless of the "immunity" of the meristem, Smith and Walker (1981) showed that the apical zone was about ten times more "infectible"

than the rest of the root of young plants of *Trifolium subterraneum*. In ericoid mycorrhizas penetration also seems to take place not in the region of the meristem, but behind it. In orchid embryos the meristem is uninfected but the developing cortical cells at or near the suspensor-end are penetrated by the fungus, and the apex of roots is uninfected.

It is arguable that the apparently greater susceptibility of the sub-apical region to penetration by the fungi gives a clue to the problem of its mechanism. In that region of a root the cells are maturing—they are increasing in size and, especially as they begin to attain their full size, their walls are being formed by the deposition of carbon polymers. It has been suggested by Harley and Smith (1983) that if the fungus inhibited the polymerizing enzymes of the host, which are building the cell-walls from soluble compounds secreted through their plasmalemma, the hyphae could penetrate the tissue without enzyme action. Penetration might be between the cells only, as in ectomycorrhiza, or also into the cells as in ectendomycorrhiza and endomycorrhiza.

Electron microscopic examination of the developing Hartig net gives the impression of penetration by mechanical means, because the leading edges are wedge shaped (Foster and Marks 1966, 1967; Hofsten 1969; Strullu 1974; Atkinson 1975; Duddridge 1980). Many, but not all, observers have described a modified host wall opposed to the hyphal wall, or to a modified hyphal wall, to form a "contact zone" or "involving" layer (Strullu 1974, 1976; Strullu and Gerault 1977; Atkinson 1975; Scannerini 1968; Duddridge and Read 1984a, b). Such modifications of the walls might arise by enzyme action altering mature walls, but they are more likely to arise from the mutual prevention of the maturation of developing cell-walls. The study of the exact position of first penetration and the examination of the state of the cell-walls in that position will help to solve the problem.

The studies of the contact zones between intracellular hyphae and the host cells in endomycorrhizas shed much light on this problem. In all cases the intracellular hypha is separated from the host by an electron-lucent zone which has been given many names, here we will call it the 'apoplastic space'. It is bounded by the wall of the hypha on the one hand, and by the plasma membrane of the host cell on the other. The process of development of the arbuscules in *Allium cepa* has been described by Dexheimer *et al* (1979). The hypha that penetrates the cell to form the main trunk of the arbuscule does not break the plasmalemma of the host which invaginates and surrounds the arbuscule and each of its branches as it develops. Even the ultimate fine branches are each enclosed separately. Between the plasmalemma and the hyphal wall is the apoplastic space. Near the point of entry of the trunk hypha, this space is occupied by an extension of the wall of the cell, but higher up this becomes thinner and gives place to an electron-lucent zone into which the plasmalemma of the host releases vesicles. In addition there are carbohydrate fibrils in the apoplastic space presumably synthesized from carbohydrate released from the vesicles. It is as if the host, as well as secreting the wall precursors in vesicles and still able to produce fibrils, is unable to organize them into a cell-wall structure. A similar zone between the plasmalemma of the host and the fungal wall is observed in all cases of mycorrhiza where active fungal hyphae occur within the lumen of physiologically active cells (see Harley and Smith 1983 for references). The continued metabolic activity of the two participants is shown by the presence in both of healthy nuclei, organelles, and cell membranes; glycogen and polyphosphate granules and lipid drops in the fungus, and increase in size of nucleus and nucleoli, and the quantity of cytoplasm in the host-cell (Strullu 1976a; Cox and Tinker 1976).

At this stage metabolically active symbionts are in contact, and it is in this phase that the bidirectional movement of substances, such as carbohydrates into the fungus and inorganic nutrients into the host must take place. In vesicular arbuscular mycorrhiza arbuscules and intracellular coils are being formed continually as the infection spreads towards the apex of the root, and they are degenerating in the more mature areas. The same is true of the hyphal coils in other endomycorrhiza and the Hartig net in ectomycorrhiza. The length of this state of contact between metabolically active symbionts is no doubt variable, but the length of life of individual arbuscules has been estimated to be between 4 and 15 days in various symbioses (Bevege and Bowen 1975).

As the arbuscule or hyphal coil begins to senesce it becomes encapsulated in cellulosic wall material. As Dexheimer *et al* (1979) suggest, it is as if the host has been prevented from producing an organized cell-wall when in contact with an active fungal hypha, but as the hypha senesces it recovers that ability. The secretion of carbohydrate which has been going on all the time continues, but now it is organized into a wall which encapsulates the ageing fungus to produce what the older observers called "digestion clumps" or "sporagioles". This process has also been described in the vesicular-arbuscular mycorrhiza of *Taxus*, and in orchid mycorrhiza by Strullu (1976). However as Bonfante-Fasolo *et al* (1981) have shown, the ericoid mycorrhiza is somewhat different, for there, after a period of contact of active hyphae and cell, the host cell degenerates and for a time active hyphae may exist in the senescent cell. Monotropoid mycorrhiza is also somewhat different, not in its earlier phases (Duddridge and Read 1982), but in its senescence. Nevertheless the consistent features of all endomycorrhizas and ectendomycorrhizas and their process of development fit well with the view put forward above that the fungus affects the enzyme activity of the host so that mature cell-wall production is prevented and hyphal penetration is made possible. It also fits the contention that the host may affect the enzymes of the fungal species that form orchid mycorrhiza. Indeed if the latter were not so, the fungus would be necrotrophic and could not be encapsulated in the final stage, in a cellulosic mass.

The manner in which their mutual interference with enzyme action comes about must have a fairly simple explanation as it is so widespread. The near universality of vesicular-arbuscular mycorrhiza amongst higher plants for instance, indicates that it must be applicable to hosts of very diverse taxonomy. Vanderplank (1978) has put forward the view, with respect to biotrophic pathogenesis, that in compatible pairs of host and pathogen the enzyme proteins produced by one, polymerize (or copolymerize) with those of the other causing inhibition of their actions. The continued synthesis of proteins by the host, which eliminates the dangerous activity of the pathogens enzymes, provides nutrient for the pathogen. Harley and Smith (1983) pointed out that this hypothesis might be adapted to the relationship of the symbionts in mycorrhizas. The carbohydrate enzymes producing the cell walls of the host would be inhibited by the proteins on the walls of the fungus (wall-attached enzymes known to exist) and as a result the hyphae could penetrate along or through the cell walls of the host in locations where the cell walls were still being built; that is behind the actual meristematic region in a zone where the cells are attaining full size. In endomycorrhizas, the cells having been penetrated, the hyphal surface-proteins similarly affect the polymerization enzymes, producing potentially encapsulating cell-walls, so that the apoplastic space has the structure of an uncompleted wall. The degeneration of the fungus allows the recovery of the host to build cell-walls and encapsulate the fungus.

Such a mechanism would also explain the control of active hydrolytic enzyme activity in mycorrhizal fungi such as those of orchid mycorrhiza.

6. Conclusion

Mycorrhizal fungi of all kinds are very loosely specific to their hosts. Host plants may form more than one kind of mycorrhiza and associate with many species of fungi of diverse taxa. The behaviour of a given fungus within the host tissues may be different in different hosts.

Penetration of the cell walls of the host does not seem to be dependent upon active hydrolytic enzymes produced by the fungal hyphae. An hypothesis is put forward that penetration occurs in regions where the host is laying down cell-walls and the fungus prevents wall development.

This essay discusses two fundamental and general aspects of the biology of mycorrhizal fungi namely their specificity to and their mode of penetration of their host. It will be seen that these mutualistic symbiotic fungi behave differently, in both these aspects of behaviour, from biotrophic and necrotrophic parasites. These subjects have been discussed in detail with Dr S E Smith during our work together.

References

Atkinson M A 1975 *The fine structure of mycorrhizas* D. Phil. Thesis, Oxford

Bevege D I and Bowen G D 1975 *Endogone* strain and host plant differences in development of vesicular-arbuscular mycorrhizas. in *Endomycorrhizas* (eds) F E Sanders, B Mosse and P B Tinker (London and New York: Academic Press) pp. 77–86

Björkman E 1960 *Monotropa hypopitys* L. an epiparasite on tree roots; *Physiol. Plant* **13** 308–327

Bonfante-Fasolo P, Berta G and Gianinazzi-Pearson V 1981 Ultrastructural aspects of endomycorrhizas in Ericaceae II. Hostendophyte relationships in *Vaccinium myrtillus*; *New Phytol.* **89** 219–224

Burgeff H 1936 *Samenkeimung der Orchideen.* G Fischer Jena

Cox G and Tinker P B 1976 Translocation and transfer of nutrients in vesicular-arbuscular mycorrhiza 1. The arbuscule and phosphorus transfer: a quantitative ultrastructural study; *New Phytol.* 77 371–378

Dexheimer J, Gianinazzi S and Gianinazzi-Pearson V 1979 Ultrastructural cytochemistry of the host-fungus interfaces in the endomycorrhizal association *Glomus mosseae/Allum cepa*; *Z. Pflphysiol.* **92** 191–206

Duddridge J A 1980 *A comparative ultrastructural analysis of a range of mycorrhizal associations*, Ph.D. Thesis, University of Sheffield

Duddridge J A and Read D J 1982 An ultrastructural analysis of the development of mycorrhizas in *Monotropa hypopitys* L; *New Phytol.* **92** 203–214

Duddridge J A and Read D J 1984a The development and ultrastructure of ectomycorrhizas. I. Ectomycorrhizal development on pine in the field; *New Phytol.* **96** 565–573

Duddridge J A and Read D J 1984b The development and ultrastructure of ectomycorrhizas, II. Ectomycorrhizal development on pine *in vitro*; *New Phytol.* **96** 575–582

Foster R C and Marks G C 1966 The fine structure of the mycorrhizas of *Pinus radiata* D. Don.; *Aust. J. Biol. Sci.* **18** 1027–1038

Foster R C and Marks G C 1967 Observations on the mycorrhizas of forest trees II; *Aust. J. Biol. Sci.* **20** 915–926

Galun M and Bubrick P 1984 Physiological interactions between the partners of the lichen symbiosis. In *Cellular interactions*, (eds) H F Linskens and J Heslop-Harrison; *Encyclop. Plant Physiol.* (Berlin, New York, Tokyo: Springer-Verlag) new series Vol. **17** 363–401.

Harley J L 1969 *Biology of mycorrhiza* (London: Leonard Hill)

Harley J L 1984 The mycorrhizal associations. In *Cellular interactions*, (eds) H F Linskens and J Heslop-Harrison; *Encyclop. Plant Physiol.* (Berlin, New York, Tokyo: Springer-Verlag) new series Vol. **17** 148–186.

Harley J L and Smith S E 1983 *Mycological Symbiosis* (London, New York: Academic Press)

Hofsten A 1969 The ultrastructure of mycorrhiza I Ectotrophic and ectendotrophic mycorrhiza of *Pinus sylvestris; Svensk. bot. Tidskr.* **63** 455–463

Laiho O 1965 Further studies on the ectendotrophic mycorrhiza; *Acta For. Fenn.* **79** 1–35

Laiho O and Mikola P 1964 Studies on the effects of some eradicants on the mycorrhizal development in forest nurseries. *Acta For. Fenn.* **77** 1–34

Last F T, Mason P A, Wilson J and Deacon J W 1983 Fine roots and sheathing mycorrhizas: their formation, function, and dynamics; *Plant Soil* **71** 9–21

Lindeberg G and Lindeberg M 1977 Pectinolytic ability of some mycorrhizal and saprophytic hymenomycetes; *Arch. Microbiol.* **101** 109–115

Linskens H F and Heslop-Harrison J (eds) 1984 Cellular interactions: a brief conspectus. In *Cellular interactions, Encyclop. Plant Physiol.* (Berlin, New York, Tokyo: Springer-Verlag) new series Vol. **17** 2–17

Marks G C and Foster R C 1967 Succession of mycorrhizal associations on individual roots of radiata pine; *Aus. For.* **31** 194–201

Mason P A, Wilson J, Last F T and Walker C 1983 The concept of succession in relation to the spread of sheathing mycorrhizal fungi on inoculated tree seedlings growing in unsterile soil; *Plant Soil* **71** 247–251

Mikola P 1965 Studies on ectendotrophic mycorrhiza of pine; *Acta For. Fenn.* **79** 1–56

Molina R J 1979 Pure culture synthesis and host specificity of red alder mycorrhiza; *Can. J. Bot.* **57** 1223–1225

Molina R J and Trappe J M 1982 Lack of mycorrhizal specificity by the ericaceous hosts, *Arbutus menziesii* and *Arctostaphylos uva-ursi*; *New Phytol.* **90** 495–509

Molina R J, Trappe J M and Strickler G S 1978 Mycorrhizal fungi associated with *Festuca* in Western United States and Canada; *Can. J. Bot.* **56** 1691–1695

Parson V and Read D J 1973 The biology of mycorrhiza in the Ericaceae. I. The isolation of the endophyte and synthesis of mycorrhizas in aseptic culture; *New Phytol.* **72** 371–379

Pérembelon M and Hadley G 1965 Production of pectic enzymes by pathogenic and symbiotic *Rhizoctonia* strains; *New Phytol.* **64** 144–151

Read D J 1974 *Pezizella ericae* sp. nov. the perfect state of a typical mycorrhizal endophyte of Ericaceae; *Trans. Br. Mycol. Soc.* **65** 381–383

Scannerini S 1968 Sull' ultrastruttura delle ectomicorrize II ultrastruttura di una micorriza di ascomycete: *Tuber albidum* × *Pinus strobus*; *Allionia* **14** 77–95

Smith S E and Walker N A 1981 A quantitative study of mycorrhizal infection in *Trifolium*: Separate determination of rates of infection and of mycelial growth; *New Phytol.* **89** 225–240

Strullu D G 1974 Étude ultrastructurale du réseau de Hartig d'une ectomycorrhize à ascomycète de *Pseudotsuga menziesii*; *C.R. Acad. Sci. Paris 278, ser. D*, 2139–2142

Strullu D G 1976 *Recherches de biologie et de microbiologie* forestieres. *Étude des relations nutrition-dévelopment et cytologie des mycorrhizas chez le Douglas* (*Pseudotsuga menziesii* Mirb) *et les Abietacées*. Doctoral Thesis, University of Rennes

Strullu D G 1976a Contribution à l'étude ultrastructurale des ectomycorrhizas a basidiomycites de *Pseudotsuga menziesii* (Mirb). *Bull. Soc. Bot. Fr.* **123** 5–16

Strullu D G 1978 Histologie et cytologie des endomycorrhizas. *Physiol. vég.* **16** 657–669

Strullu D G and Gerault A 1977 Études des ectomycorrhizas à Basidiomycetes et à ascomycètes du *Betula pubescens* (Ehr.) en microscopie electronique; *C.R. Acad. Sci. Paris 284, ser. D*, 2243–2246

Vanderplank J E 1978 *Genetic and molecular basis of plant pathogenesis* (Berlin, Heidelberg, New York: Springer-Verlag)

Warcup J H 1981 The mycorrhizal relationships of Australian orchids; *New Phytol.* **87** 371–387

Warcup J H 1971 Specificity of mycorrhizal associations in some Australian orchids; *New Phytol.* **70** 41–46

Warcup J H 1973 Symbiotic germination of some Australian terrestrial orchids; *New Phytol.* **72** 387–392

Warcup J H 1975 Factors affecting symbiotic germination of orchid seeds. in *Endomycorrhizas*, (eds) F E Sanders, B Mosse and P B Tinker (London and New York: Academic Press) 87–104

Warcup J H and Talbot P H B 1966 Perfect state of some Rhizoctonias; *Trans. Br. Mycol. Soc.* **49** 427–435

Warcup J H and Talbot P H B 1967 Perfect states of Rhizoctonias associated with orchids; *New Phytol.* **66** 631–641

Warcup J H and Talbot P H B 1970 Perfect states of Rhizoctonias associated with orchids. II; *New Phytol.* **70** 35–40

Williamson B and Hadley G 1970 Penetration and infection of orchid protocorms by *Thanatephorus cucumeris* and other *Rhizoctonia* isolates; *Phytopathology* **60** 1092–1096

Zak B 1973 Classification of ectomycorrhizas In *Ectomycorrhizae*, (eds) G C Marks and T T Kozlowski (New York and London: Academic Press) 43–78

Zak B 1974 Ectendomycorrhiza of Pacific madrone (*Arbutus menziesii*); *Trans. Br. Mycol. Soc.* **62** 202–204
Zak B 1976a Pure culture synthesis of bearberry mycorrhiza; *Can. J. Bot.* **54** 1297–1305
Zak B 1976b Pure culture synthesis of Pacific madrone ectendomycorrhizae; *Mycologia* **60** 362–369
Zak B and Marx D H 1964 Isolation of mycorrnizal fungi from roots of individual slash pine; *For. Sci.* **10** 214–227

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 111–127.
© Printed in India.

Factors affecting the occurrence of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with roots of trees

F T LAST and L V FLEMING*

Institute of Terrestrial Ecology, Bush Estate, near Penicuik, Midlothian EH26 0QB, UK

* Department of Microbiology, East of Scotland College of Agriculture, Edinburgh EH9 3JG

Abstract. The production of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with trees depends upon current supplies of host assimilates, upon host genotype and the age of the host tree, upon soil types and soil amendments, and weather.

Fruitbodies are not produced by fungi on roots severed from their tree, nor are they produced when trees are naturally or artificially defoliated. As trees, planted in 'new' sites, increase in age a succession of fungi produce fruitbodies, most arranged in annuli (rings) but some are arranged linearly, seemingly along secondarily thickened roots. From the initial 'focus', a stem base, each ring progresses outwards at rates comparable to those of grassland fairy ring fungi (10–20 cm yr^{-1}), the movement of different rings being spatially correlated. Around birch trees in brown earth soils, rings of early stage fungi (*Hebeloma crustuliniforme* and *Laccaria tortproximalis*) are followed by those of *Lactarius pubescens*, *Cortinarius* spp. and *Russula grisea*. This pattern of development is likely to be different when seedlings grow in soil permeated with mycorrhizal roots of old (living) trees as happens during natural regeneration.

Whereas more fruitbodies of *Inocybe petiginosa* were produced in association with one clone of *Betula pubescens* than with another, the reverse was true of *Laccaria tortilis*. There is evidence to suggest that soil type may influence the types of early-stage fungi producing fruitbodies. Applying lime to a Scots pine plantation decreased the production of fruitbodies by *Paxillus involutus* and *Lactarius rufus*; applying ammonium-N stimulated fruitbody production by *Paxillus involutus* but decreased that of *Amanita muscaria* and some species of *Russula*.

Seasons favouring the production of *Paxillus involutus* fruitbodies were different from those favouring *Lactarius rufus*. Fruitbody production of the late stage *Amanita muscaria*, in plantations of *Pinus patula*, was directly proportional to amounts of rainfall after an initial threshold which was smaller in old, than in young, plantations.

Keywords. Fruitbodies; fungi; mycorrhizas.

1. Introduction

Bearing in mind the worldwide (tropical and temperate) interest in the occurrence of fruitbodies of the 'larger' fungi, whether for studies of plant pathogens (including *Armillaria mellea* (Vahl.: Fr.) Kummer and *Heterobasidion annosum* (Fr.) Bref.) and fungi that decompose moribund tissues, of the evolution and systematics of fungi, the consumption of edible fungi or the sheer pleasure of collecting, it is surprising how little is known, in quantitative terms, about the factors controlling the occurrence of these structures.

As many of us know, when we stop to think about them, fungi do not occur at random. In 1946, Wilkins and Harris described a series of observations made of the fruitbodies of 'ground' fungi occurring in beech, and pine, woods in England. No fruitbodies were found from January to May: thereafter numbers steadily increased to a peak in October. These observations were followed, in due course, by those of Hora (1959) who was concerned with temporal variations between, in addition to within, seasons. In Scots pine (*Pinus sylvestris* L.) plantations he found that the seasonal

conditions that favoured fruitbody production by *Paxillus involutus* (Fr.) Fr. were not those for *Lactarius rufus* (Scop.: Fr.) Fr. and *vice versa*—*P. involutus* and *L. rufus* are both able to form mycorrhizas (Alexander 1981). Although not concerned with fungi that form mycorrhizas Parker-Rhodes (1955), theorising about the kinetics of grassland fairy-ring fungi, added another dimension to the analysis of fruitbody distribution, namely their distribution in space.

By taking records that enable changes in both spatial and temporal distributions to be elucidated, it has been possible to show that the fruitbodies of many fungi that form sheathing (ecto-) mycorrhizas with *Betula* spp., growing at sites which were previously free of trees, are spatially correlated, moving slowly outwards in successive seasons from an 'initial focus', the trunk of a tree (Ford *et al* 1980; Mason *et al* 1982). But, as indicated by Gregory (1982), there are significant differences in the biology of fungi that form 'free' and 'tethered' fairy rings. In the former, the classical grassland fairy rings attributable to species of *Agaricus*, *Marasmius* etc., the nutrient source is a 2-dimensional sheet of soil organic matter, whereas in the latter it is a 'point' source of roots whose genetical constitution is likely to differ appreciably not only between, but also within, outbreeding species of trees.

In accepting the invitation to write this paper to honour Prof. C V Subramanian's retirement, we decided to assess our knowledge of the factors determining the occurrence of fruitbodies of sheathing mycorrhizal fungi—'tethered' fungi. In doing so, however, we recognize that we are restricting our attention to only one aspect, reproductive, of fungal development which ideally should not be divorced from antecedent, and continuing, vegetative growth.

2. Host dependence

By searching the records, it has been possible to compile a list of the genera of 'larger' fungi associated with trees in India (table 1). While accepting that the list is likely to be extended as interest in trees increases, there is a remarkable similarity with the list

Table 1. Genera of larger fungi (agaric and bolete) that have been recorded producing fruitbodies in association with trees in India (Manjula 1983; Natarajan and Raman 1983).

Agaricus	Laccaria
*Amanita	*Lactarius
Armillaria	*Leccinum
*Boletus	Lepiota
*Cantharellus	Lepista
Clitocybe	Mycena
Collybia	*Naucoria
*Cortinarius	Oudemansiella
*Gomphidius	
*Hebeloma	*Russula
Hygrophorus	*Suillus
Hypholoma	*Tricholoma
*Inocybe	*Xerocomus

* Genera with species proven or suspected of being able to form sheathing mycorrhizas with roots of trees.

prepared by Watling (1984) for birch woodlands in the UK. The list for India, like that for the UK, includes fungi that are regarded as litter decomposers, whereas others have been proven, or are suspected of being able, to form sheathing mycorrhizas. When Rommell (1938) dug trenches to isolate root-containing plots (varying in surface area from 6 to 52 m^2) from nearby specimens of Norway spruce, *Picea abies* (L.) Karst, he found that the production of fruitbodies of sheathing mycorrhizal fungi (species of *Cantharellus*, *Cortinarius*, *Lactarius* and *Suillus*) was halted, whereas that of non-mycorrhizal fungi continued (table 2). Laiho (1970) similarly found that the development of *Paxillus involutus* fruitbodies was arrested when the organic links between fruitbodies and their 'host' trees were severed. Because fruitbody growth sometimes continued for a few days, particularly when they were developing lm or more from the zone of severance, Laiho suggested that the cessation of fruiting was attributable to the exhaustion of supplies of host (tree)-derived energy. Lewis (1973) classified mycorrhizal fungi as ecologically obligate symbiotic biotrophs: in other words, while aiding the growth of their hosts, mycorrhizal fungi are, at the same time, host dependent, the most obvious form of this dependency being related to the production of fruitbodies. While the observations made by Rommell and Laiho were usually unequivocal, on occasions fruitbodies of mycorrhizal fungi appeared afresh in ground that had been isolated, as also happened in some of Fleming's (1984) experiments. In these instances fruitbody production was attributable to their development on mycorrhizal roots that either had not been completely severed or had re-invaded the isolated areas.

Instead of directly interfering with roots Hacskaylo (1965) influenced their supplies of assimilates by manipulating foliage. He found that shading or decapitating Virginia pine, *Pinus virginiana* Mill, halted the production of earthfans of *Thelephora terrestris* (Ehrh.) Fr. By defoliating *Betula* spp., in the late summer/autumn, Last *et al* (1979) found that the production of fruitbodies of a wide range of sheathing mycorrhizal fungi (including *Hebeloma crustuliniforme* (Bull: St. Amans) Quélet, *H. mesophaeum* (Pers:

Table 2. Effects of severing tree roots on the production of fungal fruitbodies in a woodland dominated by Norway spruce (*Picea abies*) (Rommell 1938).

	Presence (+) or absence (−) of fruitbodies	
	within areas isolated by trenching—roots are not linked to their parent trees	in areas outside those isolated by trenching—roots are organically linked to parent trees
I. Fungi known, or suspected, to be capable of forming sheathing mycorrhizas.		
Cantharellus cibarius Fr.	−	+
Cortinarius cinnamomeus (L.: Fr.) Fr.	−	+
Lactarius glyciosmus (Fr.: Fr.) Fr.	−	+
L. rufus (Scop.: Fr.) Fr.	−	+
Suillus (Boletus) variegatus (Fr.) O. Kuntze.	−	+
II. Fungi known, or suspected, to be incapable of forming sheathing mycorrhizas.		
Calocera viscosa (Pers.: Fr.) Fr.	+	+
Entoloma sericeum (Bull.: Mérat) Quél.	+	+
Galera hypnorum (Schrank: Fr.) Kühn	+	+

Fr.) Quélet, *H. saccharíolens* Quélet, *Inocybe lanuginella* (Schroet.) Konrad. & Maubl., *Laccaria* spp., *Lactarius pubescens* (Fr.: Krombh.) Fr. and *Leccinum scabrum* (Fries) S. F. Gray), was adversely affected (figure 1). When trees were completely defoliated at the end of August, fruitbody production was halted for the season. However, when defoliated one month earlier (late July), lateral buds started to break and develop with the production of a new flush of leaves in September, an event accompanied by the recommencement of fruitbody production by mycorrhizal fungi—a clear indication of the close dependent link between trees and mycorrhizal fungi. However, it would be unwise to make generalisations about different species of fungi as Lundeberg (1970) found that some isolates of *Xerocomus* (*Boletus*) *subtomentosus* (L.: Fr.) Quél, were independent saprotrophs, unable to form sheathing mycorrhizas, whereas other isolates were host dependent mycorrhiza formers. Also, Giltrap (1981) observed that some mycorrhiza-forming *Boletus* spp. were able to produce fruitbodies in pure culture in the absence of their (tree) hosts.

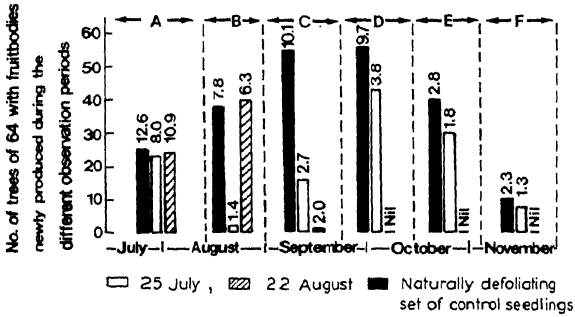


Figure 1. Mean effects of defoliating *Betula* spp. on either 25 July or 22 August on numbers of replicate trees with fruitbodies of associated mycorrhizal fungi newly produced within the different periods of observation. (Figures above the columns are mean numbers of fruitbodies per tree produced during each period; they were calculated from replicates producing at least one new fruitbody during the relevant period) (Last *et al* 1979).

3. Temporal distribution

There are many reports in the literature suggesting that mycorrhizal associates do not remain the same throughout the life of a tree. Mikola (1965) found that the ectendomycorrhizas occurring on roots of Norway spruce growing in nursery soils were substituted by sheathing (ecto-) mycorrhizas after being transplanted into forests. Lamb (1979), likewise, found that numbers of species, or putative species, of fungi forming sheathing mycorrhizas with Monterey pine, *Pinus radiata* D. Don were appreciably greater on old, than on young trees. Not surprisingly, therefore, observations of fruitbodies have shown a similarly increasing diversity: Miller (1983), in Idaho, USA, recorded the fruitbodies of five species of mycorrhizal fungi associated with stands, 15 years-old, of western white pine (*Pinus monticola* Dougl. ex D. Don) with numbers of species increasing to 34/37 and 98 in stands 30–40 and 175–215 years-old respectively.

Since 1979, Chu Chou and her associates in New Zealand have reported a series of detailed observations of Monterey pine, Douglas fir (*Pseudotsuga menziesii* (Mirb.)

Franco and *Eucalyptus* spp. Starting with Monterey pine she found numerous fruitbodies of *Hebeloma crustuliniforme*, *Laccaria laccata* (Scop.: Fr.) Cke and two species of *Rhizopogon* (*R. rubescens* and *R. luteolus*), in forest nurseries. However, while the others continued to produce fruitbodies for 3 to 5 years after transplanting Monterey pine seedlings into plantations *H. crustuliniforme* ceased production. In due course *L. laccata* and *Rhizopogon* spp. were joined and superseded by fruitbodies of species of *Inocybe* and *Suillus* which in turn were joined, when the trees were more than 10 years old, by those of *Amanita muscaria* (L.: Fr.) Hooker and *Scleroderma verrucosum* Vaill.: Pers. (Chu Chou 1979). With Douglas fir, Chu-Chou and Grace (1981) recorded a virtually identical set of fruitbody occurrences to those detailed for Monterey pine. *H. crustuliniforme* was similarly restricted to the nursery phase while *A. muscaria* was only associated with stands more than 13 years old. Similar sequences of fruitbodies were found in ageing plantations of *Eucalyptus* (5 species were observed—*E. delegatensis*, *E. fastigata*, *E. nitens*, *E. regnans* and *E. saligna*), but the species of fungi tended to differ (Chu Chou and Grace 1982). Thus *Hydnangium carneum* Wallr., *Scleroderma* spp. and *Laccaria laccata* were found in nurseries, whereas *Hebeloma crustuliniforme* and *Rhizopogon* spp. were not. Soon after transplanting fruitbodies of species of *Hymenogaster* and *Hysterangium* began appearing, to be joined later by those of species of *Cortinarius*, *Inocybe*, *Lycoperdon* and *Tricholoma*.

The observations made by Mikola, Lamb, Miller and Chu Chou and her associates all confirm that the diversity of mycorrhizal fungi associated with trees increases as trees get older but, because of the switch from nursery to forest soils, it is impossible to assess the part played, at least during the early years, by changes of soil type. Are the early changes in fruitbody assemblages attributable to changes of soil environments and/or to host factors associated with increasing age? To an extent this question has been answered by observations made on a stand of *Betula* spp. at the Bush Estate about 10 km south of Edinburgh, Scotland (lat. 55°52'N). Surface sterilised seeds were germinated and propagated in partially sterilised compost before being transplanted in November 1971 from a glasshouse to a brown earth field site where the developing trees have remained ever since (Mason *et al* 1982; Last *et al* 1984a). As with Monterey pine and Douglas fir the early appearances of fruitbodies of *Hebeloma crustuliniforme* and *Laccaria* sp. (now thought to be *L. tortilis* ([Bolt.] S. F. Gray) Cke.) were soon followed by the earthfans of *Thelephora terrestris* (in year 3) and the toadstools of *Inocybe lanuginella* and *Lactarius pubescens* in year 4 with species of *Cortinarius* and *Leccinum* appearing in year 6 and those of *Russula* spp. in year 10. The sequential changes associated with one of the replicate trees showed that the production of *H. crustuliniforme* fruitbodies ceased between years 7 and 10, when those of *H. fragilipes* Romagnesi were abundant. While fruitbodies of *Lactarius pubescens* were not formed in year 4 they occurred in year 7 and thereafter, such that in year 12 most fruitbodies associated with this particular tree were attributable to *Lactarius pubescens*. *Cortinarius* sp. and *Russula grisea* (Pers.: Secr.) Fr. (figure 2).

Although there are differences in soil types, observations made by Dr J Miles (personal communication) suggest how this succession may develop. He found that most of the mycorrhizal fruitbodies in a 20 year-old northern temperate stand of silver birch, *Betula pendula* (Roth.) were attributable to *Cortinarius* spp. and *Lactarius pubescens*, whereas those of *Amanita muscaria*, *Tricholoma columbetta* (Fr.) Kummer, *Laccaria amethystina* (Bolt.: Mérat) Murr. and *Lactarius tabidus* Fr. were the commonest in a similar stand 72 years old.

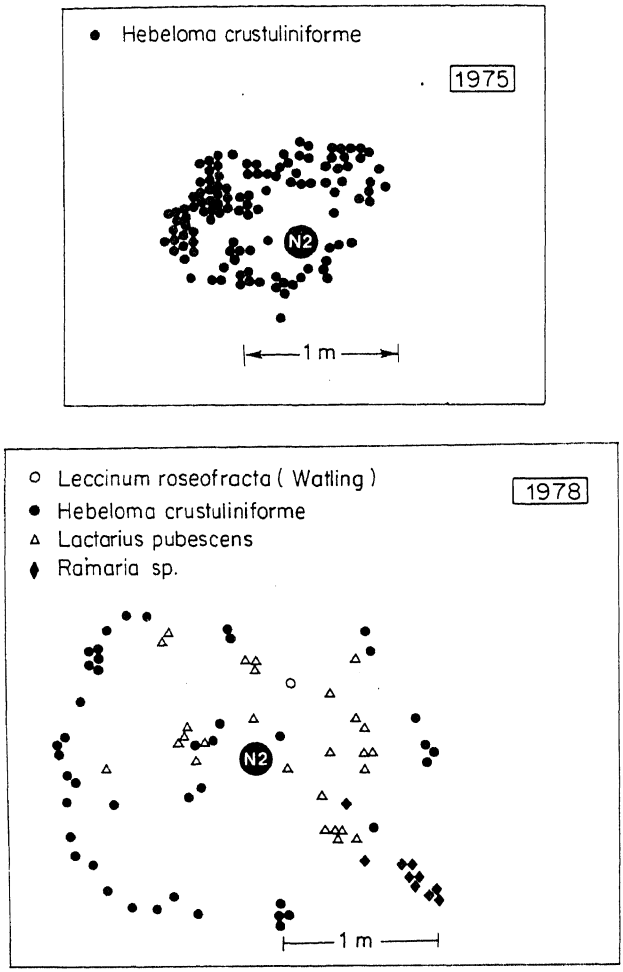


Figure 2.

Because the sequence of fruitbodies was not vitiated by the change from nursery to forest soils that occurred after the onset of fruitbody formation in the sequences recorded by Chu-Chou and her associates, the observations made on the ageing stand of birch can be regarded as illustrating the way in which the production of fruitbodies of mycorrhizal fungi is dependent on the age of their (tree) hosts. Thus it can be argued that the production of fruitbodies of *Hebeloma crustuliniforme*—an early-stage fungus—observed by Chu Chou in association with nursery seedlings of Monterey pine and Douglas fir, would have ceased because of the increasing age of the seedling trees at, or soon after, planting into forests. However, as indicated later in the section dealing with soils and soil amendments, the types of early-stage fungi may be influenced by soil, in addition to host factors—there is evidence of a fungus × soil interaction.

There is, however, reason to believe, that the fruitbody sequences developing in association with saplings regenerating among older, mature trees may be conspicuously different from those just described in association with seeds or saplings planted at sites which had previously been freed of living trees, as in nursery seed-beds and clear-felled

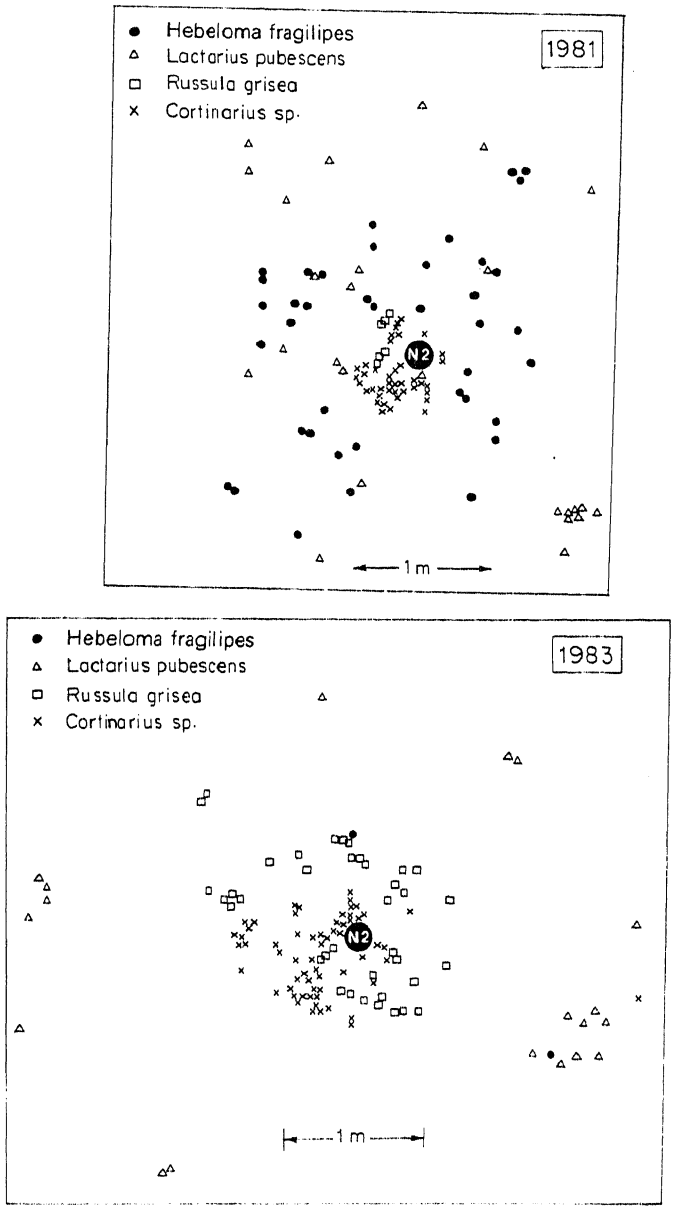


Figure 2. Positions of fruitbodies produced in late summer/autumn of 1975, 1978, 1981 and 1983 around a specimen (N2) of silver birch, *Betula pendula*, planted in a brown earth in 1971 from seed germinated in 1970 (Last *et al* 1984a).

locations. Fleming (1984) found that mycorrhizas of *Lactarius pubescens* (not regarded as an early-stage fungus) developed on seedlings planted into a woodland soil permeated by intact roots of relatively old trees with mycorrhizas of *L. pubescens*, but they didn't develop when the roots of the old trees had been severed. Thus, it seems that *L. pubescens* and probably other late-stage fungi (*sensu* Deacon *et al* 1983) depend, at

least to a degree, on host-derived assimilates for sufficient inoculum potential to colonize seedling roots (Fleming 1984). When this potential is available, as is likely to be the case when seedling trees naturally regenerate in, or at the periphery, of mature woodlands, the sequences described in New Zealand and Scotland may be altered.

The concept of a succession, as applied to sheathing mycorrhizal fungi, is based virtually exclusively upon observations made on trees growing in temperate regions, both north and south of the equator. The few observations made in the tropics suggest that the timescale of successions, if they exist, may be considerably shorter. When observing the occurrence of the fly agaric *Amanita muscaria* in the Palni Hills near Kodaikanal (lat. 10°15'N; long. 77°31'E), southern India a few of its fruitbodies were associated with plantations of *Pinus patula* as young as 5 years-old, its numbers thereafter increasing from 2·7 to 310 per 1000 trees per month in year 16 (figure 3) (Last *et al* 1981).

In discussing the factors affecting fruitbody production by fungi colonizing dung, Harper and Webster (1964) suggested that the sequence may simply reflect shorter or longer periods of fruitbody development required by different fungi. Interestingly Deacon *et al* (1983) found that species of early stage fungi, associated with birch, tend to produce lighter (air dried) fruitbodies (*Hebeloma crustuliniforme*, 0·35 g; *Inocybe lanuginella* 0·16 g) than fungi appearing in later years (*e.g. Lactarius pubescens*, 2·23 g; *Leccinum* spp. 5·03–10·59 g). Thus the early stage fungi may be less demanding of their hosts than late stage fungi, a suggestion in keeping with the idea that a tree has a limited capacity, which may change with age, to support fruitbody production.

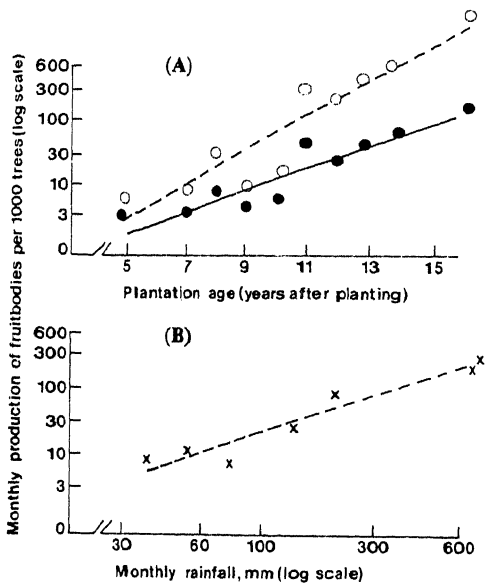


Figure 3. Relationships during 1978 between the monthly production of *Amanita muscaria* fruitbodies and **A.** the ages of different plantations of *Pinus patula* (•, regressions calculated with data set February/December; o, regressions calculated after excluding data for the months without rain, *viz.* February, March, June and August) and **B.** monthly amounts of rain (---- regression restricted to data for the seven months with rain) (Last *et al* 1981).

4. Spatial distribution

Diagrams prepared by Horn (1933) and Becker (1956) show that the fruitbodies of, among others, *Hebeloma crustuliniforme* (associated with sweet birch, *Betula lenta* L.) and *Suillus granulatus* (Fr.) O. Kuntze, *Lactarius deliciosus* (L.: Fr.) S. F. Gray, *Hygrophorus hypothejus* (Fr.: Fr.) Fr. and *Tricholoma albobruneum* (Pers.: Fr.). Kummer do not occur at random around Scots pine. Instead they occur in perceptible rings or annuli as do grassland 'fairy ring' fungi. However, unlike those of grassland 'fairy ring' fungi, the fruitbodies of mycorrhizal fungi are sometimes significantly clumped within the rings with the simultaneous 'development' of gaps (Ford *et al* 1980). Is it conceivable that this patchiness results from the activity of fungal self inhibitors or the absence of mycorrhizas? Certainly the development of succeeding generations, 'flushes', of *Agaricus bisporus* (Lange.) Pilat mushrooms is restricted if mature fruitbodies are not removed (Cooke and Flegg 1965).

With some exceptions, notably *Laccaria tortilis* (Mason *et al* 1982) and species of *Lycoperdon* and *Scleroderma* (author's observations), fruitbodies of mycorrhizal fungi occur in rings whose radii, like those of grassland 'fairy rings', increase from year to year. In the second year after planting 60 birch saplings into a brown earth field soil the fruitbodies of *Hebeloma crustuliniforme* were concentrated into rings, encircling stem bases, with a mean radius of 22 cm which by year 6 had increased to 73 cm. The rings of *Lactarius pubescens* which first appeared in year 4, with a mean radius of 40 cm, moved progressively outwards such that in year 6 their mean radius was 69 cm. Thus, as suggested by Ford *et al* (1980), arrays of mycorrhizal fruitbodies show a slow, but spatially correlated, outward movement from the stems of trees. In another example, the mean radii of *H. crustuliniforme* around a specimen of silver birch increased in the period 4 to 7 years after planting, by 15·3 cm yr^{-1}; those of *L. pubescens* spread 24·8 cm yr^{-1} between years 7 and 12 while those of *Cortinarius* sp. and *Russula grisea* (Pers. ex Secr.) Fr. increased by 7 and 17 cm yr^{-1} respectively (table 3). Thus, there appear to be considerable differences in the rates of advance of rings of 'tethered' mycorrhizal fungi (*sensu* Gregory 1982), comparable to those occurring among 'free' grassland fungi such as *Marasmius oreades* (Bolt. ex Fr.) Fr. (Dickinson 1979).

In summary, the distributions of fruitbodies of mycorrhizal fungi may vary in time and space (figure 4) and inevitably it is likely that an annulus occupied by a fungus one

Table 3. Numbers and spatial distribution of the fruitbodies of different sheathing mycorrhizal fungi occurring in association with *Betula pendula* in different seasons after planting (Last *et al* 1984a).

Years after planting	*Hebeloma crustuliniforme*	*Lactarius pubescens*	*Ramaria* sp.	*Hebeloma fragilipes*	*Cortinarius* sp.	*Russula 'grisea'*
4 (1975)	115 (*47*)	Nil	Nil	Nil	Nil	Nil
7 (1978)	41 (*93*)	27 (*58*)	11 (*96*)	Nil	Nil	Nil
10 (1981)	Nil	26 (*120*)	Nil	40 (*84*)	39 (*27*)	6 (*27*)
12 (1983)	Nil	19 (*182*)	Nil	2 (N.A.)	58 (*41*)	37 (*61*)

Italicised numbers in brackets = mean radial distances (cm) of fruitbodies from base of stem of specimen of *Betula pendula*.

N.A.—too few fruitbodies to enable a valid mean radial distance to be calculated.

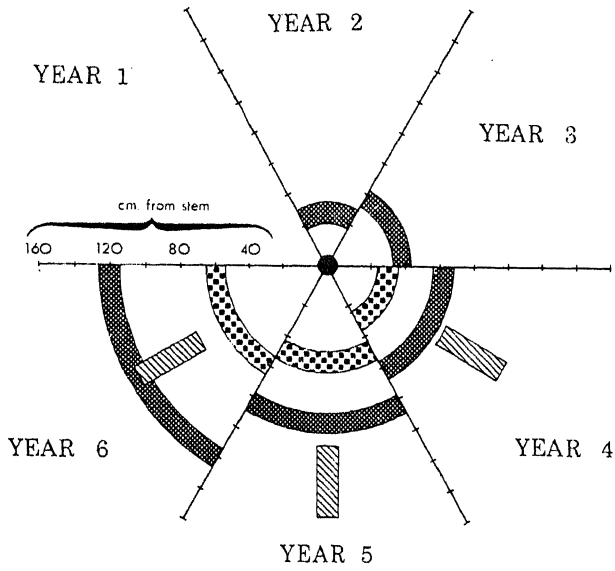


Figure 4. Mean spatial distributions around the stems (●) of silver birch (*Betula pendula*) of fruitbodies of sheathing mycorrhizal fungi appearing during the first six years after planting (Last *et al* 1983).

year may be occupied by another fungus in subsequent years. In 1977 Ford *et al* (1980) found that fruitbodies of *Lactarius pubescens* were a similar mean distance from a specimen of *B. pendula* as those of *H. crustuliniforme* had been two years earlier (figure 5). However, although the two fungi were at similar distances from the same trees, they mostly did not occupy the same positions within the 'shared' annulus.

5. Effects of host genotypes

In an experimental stand of birches, south of Edinburgh (lat. 55°52′N), there was an inverse relation between the numbers of fruitbodies, of mycorrhizal fungi, produced per tree per year and the distances of the different seed sources from the equator (Mason *et al* 1982). In the first 2 years after planting fruitbodies were associated with all replicate saplings grown from seed collected at latitudes between 50° and 54°59′N, yet no fruitbodies were associated with saplings from more northerly latitudes until year 3 (lat. 55°–64°59′) or year 6 (lat. 65°–69°59′). In year 5 there were 150, 53 and nil fruitbodies associated with trees from lat. 50°40′N (southern England), 57°20′N (Latvia) and 66°30′N (northern Sweden) respectively, this difference in numbers being reflected in comparable differences in fungal diversity. In year 6, there were three species of fungi associated, for the first time, with saplings from 66°30′N—(*Hebeloma*

Figure 5. Distributions of fruitbodies of two mycorrhizal fungi, *Hebeloma* spp. and *Lactarius pubescens* found in association with silver birch, *Betula pendula*. Data for *Hebeloma* spp. and *L. pubescens* were recorded 5 and 7 years after planting respectively. (Ford *et al* 1980).

crustuliniforme, *Hebeloma* sp. and *Inocybe lanuginella*) whereas there were 10 associated with saplings from 50°41'N (*Cortinarius* sp., *H. crustuliniforme*, *H. fragilipes*, *H. mesophaeum*, *H. sacchariolens*, *Inocybe lanuginella*, *Laccaria laccata*, *L. proxima* (Boud.) Pat., *Lactarius pubescens* and *Leccinum scabrum*).

These effects of seed sources on the production of fruitbodies are linked with the rates of autumnal foliar yellowing—trees from the 'unproductive' northernmost latitude 66°30'N were completely defoliated in mid-August whereas the 'productive' trees from southern England retained photosynthetic foliage until late September (figure 6)—an effect which has been mimicked experimentally (figure 1). However, the differing responses of vegetatively propagated clones, with similar patterns of leaf retention, cannot be explained in this way. When two silver birch seedlings, from the same seed collection, were used as stock plants for the vegetative production of cuttings (Last *et al* 1984b) it was found that there were usually 20 fruitbodies per year associated with one clone (12·5D) but only 8 fruitbodies with the second clone (V21H). A similar difference was found between two clones of downy birch, *Betula pubescens* Ehrh.; 11

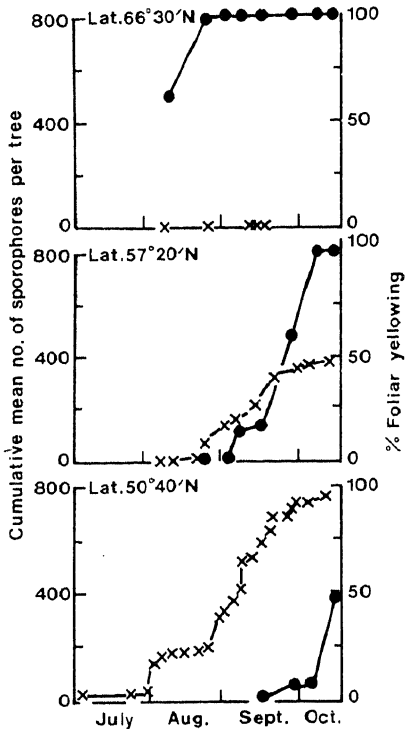


Figure 6. Patterns during 1977 (6 years after planting) of seasonal leaf yellowing (•) and the occurrence of fruitbodies (×) of sheathing mycorrhizal fungi associated with silver birch, *Betula pendula*, when saplings from seeds collected at three different latitudes were grown at latitude 55°52'N (Bush Estate, near Edinburgh) (Last *et al* 1979).

fruitbodies were associated with one genotype (9·3G) compared with 5 fruitbodies associated with another (9·3D). In this instance, however, there was also a significant effect on the array of fungi producing fruitbodies. Significantly more fruitbodies of *Inocybe petiginosa* (Fr.: Fr.) Gillet. were associated with 9·3D than with 9·3G whereas the reverse was true of *Laccaria tortilis*—the locally abundant two-spored species of *Laccaria*. These observations, while not discounting possible microsite effects, suggest that the different assemblages of fruitbodies associated with neighbouring trees, of the same outbred populations, may reflect the effects of different host genotypes.

6. Effects of weather/climate

In temperate areas fruitbody production is limited by rainfall and temperature (Wilkins and Harris 1946). While all fungi require adequate amounts of moisture for the formation of their fruitbodies, responses to temperature may show greater variability. Wilkins and Harris considered that minimum temperatures, *per se*, were more restrictive than maximum temperatures, the former directly restricting fruitbody development whereas the effects of the latter are likely to be associated with soil

moisture deficits. For this reason, and because mycorrhizal fungi depend on the current products of host photosynthesis for fruitbody formation, periods of toadstool formation are likely to be restricted to the period between the onset of warm and moist conditions and the completion of leaf abscission in stands of deciduous trees and between the onset of moist conditions and the inhibition of fruitbody expansion by restrictive cold temperatures in stands of evergreen trees. Even so, Hora (1959) found that the seasonal production of *Paxillus involutus* fruitbodies in stands of Scots pine was strongly bimodal with peaks in mid September and late October, whereas that of *Lactarius rufus* had a single peak in mid September. Mason *et al* (1982) found fruitbodies of *Hebeloma sacchariolens* newly emerging in a young stand of *Betula* spp. over a period of 40 days from 12 August; 80% emerged between 25 August and 15 September. In contrast those of *H. crustuliniforme* emerged between 24 August and 4 October with 80% developing between 12 and 21 September, while 80% of the late developing (8–27 September) *H. fragilipes* emerged on either the 20th or 21st September. *Lactarius pubescens*, like *H. crustuliniforme*, produced 80% of its fruitbodies over a period of 12 days in this instance 26 August to 7 September, but *Inocybe lanuginella* and a species of *Laccaria*, probably *L. tortilis*, were productive over much longer periods—4 July to 21 September and 2 August to 12 October respectively.

At this stage it is appropriate to be reminded that the temperature optima for the vegetative and reproductive phases of the non-mycorrhizal cultivated mushroom *Agaricus bisporus* are different, 24° and 15°C respectively (Flegg 1968); also that fruitbody production is influenced by (i) the associated bacterium *Pseudomonas putida*, (ii) the local concentrations of carbon dioxide and (iii) substrate moisture. Of these factors, only moisture seems to have been investigated in relation to mycorrhizal fungi, although Watling (1974) suggested that the appearance in Scotland, after hot summers, of species more typical of southern England may have been due to the warmer than average temperatures 'triggering' the initiation and/or expansion of fruitbody primordia. Last *et al* (1981) examined the relationship between (i) monthly rainfall (R) in mm and (ii) the monthly production of fruitbodies of *Amanita muscaria* (N = numbers of fruitbodies 1000 trees^{-1}) associated with plantations of *Pinus patula* Schl. and Cham. at Kodaikanal, Tamil Nadu, India (altitude 2200 m; latitude 10°15'N)—they proved to be directly proportional.

$$\log_e (N+1) = 3{\cdot}65 + 0{\cdot}37\,A + 0{\cdot}75 \times \log_e (R+1)$$

where A = age of plantation, years after planting (figure 3).

There was, however, a significant interaction between the effects of rainfall and plantation age. Whereas the production of *A. muscaria* fruitbodies was triggered in 'old' plantations by smaller amounts of rain than were needed in young stands, the subsequent responses to increasing amounts of rain were larger in young, than in old, stands. Thus, in months with rain, an increase in rainfall from 100 to 300 mm increased the production of *A. muscaria* fruitbodies in young plantations (5–7 years old) by a factor of × 6 from 3·2 to 20 fruitbodies 1000 trees^{-1} and in plantations 15 to 17 years old by a factor of × 3·3 from 330 to 1100 (Last *et al* 1981). In temperate conditions in the UK Hering (1966) considered that the mycorrhizal *Russula emetica* (Schaeff.: Fr.) S. F. Gray in a broad-leaved woodland produced fruitbodies more rapidly in response to rain than *Laccaria amethystea* (Bull.: Mérat) Murrill, neither of which was as sensitive, or responsive, as the litter decomposing *Mycena galopus* (Pers.: Fr.) Kummer.

7. Effects of soil and soil amendments

While there are many records of the abundance or otherwise of toadstools of different mycorrhizal fungi associated with the same tree species growing in different soils, there is a dearth of information isolating the effects of soils from those of other environmental variables. However, when three clones of *Betula* spp. were propagated in groundwater gleys with mull humus with either smaller (soil A) or larger (soil B) amounts of silt and clay, before being transplanted to a field of soil B, it was found that the different propagating soils had profound effects on the numbers and types of mycorrhizal fruitbodies produced in the ensuing six years (Last *et al* 1984a). On average there were 19 fruitbodies per tree per year associated with trees propagated in soil A and only 4 in soil B. While this difference is mainly attributable to the presence or absence of *Laccaria tortilis*, a fourth *Betula* clone 'produced' less than one toadstool of this fungus, per tree per year, in either soil. There is, therefore, evidence of a host × soil interaction (table 4).

Soil amendments may also influence the production of mycorrhizal fruitbodies. Hora (1959) found that the application of some fertilizers, ammonium sulphate, superphosphate or a complete fertilizer 'Growmore', increased the production of fruitbodies by *Paxillus involutus* and *Lactarius rufus* when applied to plantations of Scots pine, the ammonium form of nitrogen appearing to be more stimulatory than the nitrate form. By contrast, adding lime decreased fruitbody production by *P. involutus*

Table 4. Effects during 1978 of 'host' genotypes and types of propagating soils on total numbers of fruitbodies of mycorrhizal fungi, also separately of *Inocybe petiginosa* and *Laccaria tortilis*, found in association with *Betula* spp. planted 3 years previously (numbers of fruitbodies per tree, *n*, transformed to log $(n+1)$ for statistical analyses) (Last *et al* 1984b).

Types of propagating soil	Host genotypes: Clones of *B. pubescens*		Clones of *B. pendula*	
	9·3D	9·3G	V21H	12·5D
I. Total numbers of fruitbodies per tree (LSD ($p = 0{\cdot}05$) = *0·31*)				
A	5·0	19·0	23·0	43·7
	(*0·78*)	(*1·30*)	(*1·38*)	(*1·65*)
B	5·9	4·8	3·0	2·4
	(*0·84*)	(*0·76*)	(*0·60*)	(*0·53*)
II. Numbers of fruitbodies of *Laccaria tortilis* (LSD ($p = 0{\cdot}05$) = *0·23*)				
A	0·3	15·5	15·9	37·3
	(*0·13*)	(*1·22*)	(*1·23*)	(*1·58*)
B	0·2	0·4	0·2	0·2
	(*0·06*)	(*0·16*)	(*0·08*)	(*0·07*)
III. Numbers of fruitbodies of *Inocybe petiginosa* (LSD ($p = 0{\cdot}05$) = *0·19*)				
A	3·3	0·5	<0·1	<0·1
	(*0·64*)	(*0·15*)	(*0·03*)	(*0·03*)
B	5·3	1·4	0·4	0·5
	(*0·80*)	(*0·38*)	(*0·15*)	(*0·17*)

and *L. rufus*. Shubin *et al* (1977) distinguished, by their responses to the application of nitrogenous fertilisers, three groups of mycorrhizal fungi in a 30-year-old birch stand: fruitbody production was (i) decreased (*Amanita muscaria*, and some species of *Cortinarius* and *Russula*), (ii) unaltered (some species of *Lactarius*, *Tricholoma* and *Xerocomus*) or (iii) increased, notably *Paxillus involutus* as found by Hora.

8. Discussion

From his study of a plantation of Scots pine in Scotland, Richardson (1970), averaging data for five years, estimated that 0·24 to 0·49 million fruitbodies, of agarics and boletes, were produced annually per hectare: in terms of biomass they were equivalent to 265–460 kg (fresh weight) $ha^{-1}yr^{-1}$. These amounts are of a similar range to those estimated for coniferous forests in Finland and the USA (Rautavaara 1947), but are appreciably larger than the fresh weights of comparable fungi in broadleaved woodlands (see Hering 1966). None of these authors separately estimated the weights of fruitbodies attributable to either (a) sheathing mycorrhizal or (b) saprotrophic fungi. However, Harley (1971) reckoned that the diversion of tree assimilates to the production of fruitbodies of mycorrhizal fungi (also the Hartig net within roots, the 'covering' fungal sheath and the hyphae ramifying soil) may represent a significant drain on the resources of the host trees. In some circumstances the drain might be equivalent to one cubic metre of timber ha^{-1} although this amount, calculated by P Larsen (see Rommell 1939) seems likely to be an overestimate.

In making this review, the dependence of mycorrhizal fungi upon their hosts has been stressed. However it is important to remember that the observations do not differentiate between the stimuli essential for the (a) initiation and (b) subsequent enlargement and extension of fruitbody primordia. Although there are reports that the above-ground appearance of fruitbodies is usually reflected below-ground by the occurrence of relevant mycorrhizas (Deacon *et al* 1983), searches have not been made systematically of the 'spaces' between fruitbodies. It may be that the absence of fruitbodies from locations where mycorrhizas are known to occur indicates that other conditions may be inappropriate for primordia initiation and/or enlargement. Whatever the reason or reasons it seems that fruitbody production is sensitive to environmental conditions, *e.g.* weather.

In addition to distinguishing decomposer, from mycorrhizal, fungi—the former unlike the latter continue to produce fruitbodies on blocks of soil whose complement of roots has been severed from nearby trees—it is also possible within mycorrhizal fungi to distinguish groups based on their production of fruitbodies and their abilities to colonize seedlings. Both groups are able to form mycorrhizas on seedlings in axenic 'sterile' conditions but only one group termed early-stage including *Hebeloma crustuliniforme* and *Inocybe lanuginella* (*sensu* Deacon *et al* 1983), whose fruitbodies are more commonly associated with young trees, are able to colonize seedlings in unsterile conditions from basidiospores. The other group, late-stage fungi, including *Lactarius pubescens* and *Russula grisea*, do not have this ability (see summary of Fox's data in Mason *et al* 1984) and the available evidence suggests that these fungi depend, at least to a degree, on host-derived assimilates for sufficient inoculum potential to colonize seedling roots (Fleming 1984). The role of fruitbody, and spore, production in the biology of late-stage fungi is therefore unclear. However, at some stage colonization by

germinating spores must occur: it may be associated with the changes in root physiology and associated rhizosphere microbes that occur as trees increase in age.

Using the terminology adopted in studies of population behaviour, Dighton and Mason (1985) suggested that early-stage fungi can be regarded as having similarities to '*r*-species (those that spend most of their time in acts of colonization and then give way to others in natural succession)', whereas late-stage fungi are more nearly related to '*K*-species (specialists in resource-limited environments where there is intense interference from neighbours)' (Harper 1977). Interestingly early-stage fungi such as *Laccaria tortilis* produce large numbers of small fruitbodies whereas late-stage fungi, *K*-species, such as species of *Amanita* and *Leccinum*, instead seem to use their host-derived assimilates to produce a relatively small number of much larger fruitbodies.

When planning this contribution we intended to write about the 'demography' of the fruitbodies of fungi forming sheathing mycorrhizas. However, for completeness, this would have necessitated a discussion of vegetative, in addition to reproductive, growth. At present we know little of the factors controlling the vegetative growth of mycorrhizal fungi and, as this review illustrates, our knowledge of the factors controlling fruitbody (and spore) production is undesirably incomplete. Is it unrealistic to hope that mycologists will, in future, devote more of their time to repeated observations of the occurrence of fruitbodies at an inevitably restricted array of sites—the switch from surveying to monitoring might prove to be unexpectedly rewarding as suggested by Richardson (1970).

Acknowledgement

The authors wish to acknowledge the help of Mr R C Munro who prepared the figures.

References

Alexander I J 1981 The *Picea sitchensis* + *Lactarius rufus* mycorrhizal association and its effects on seedling growth and development; *Trans. Br. Mycol. Soc.* **76** 417–423

Becker G 1956 Observations sur l'ecologie des champignons superieurs; *Ann. Sci. Univ. Besancon* **7** 15–128

Chu-Chou M 1979 Mycorrhizal fungi of *Pinus radiata* in New Zealand; *Soil Biol. Biochem.* **11** 557–562

Chu-Chou M and Grace L J 1981 Mycorrhizal fungi of *Pseudotsuga menziesii* in the north island of New Zealand; *Soil Biol. Biochem.* **13** 247–249

Chu-Chou M and Grace L J 1982 Mycorrhizal fungi of *Eucalyptus* in the north island of New Zealand; *Soil Biol. Biochem.* **14** 133–137

Cooke D and Flegg P B 1965 The effect of stage of maturity at picking on the flushing of crops of the cultivated mushroom; *J. Hortic Sci.* **40** 207–212

Deacon J W, Donaldson S D and Last F T 1983 Sequences and interactions of mycorrhizal fungi on birch; *Plant Soil* **71** 257–262

Dickinson C H 1979 Fairy rings in Norfolk; *Bull. Br. Mycol. Soc.* **13** 91–94

Dighton J and Mason P A 1985 Mycorrhizal dynamics during forest tree development In: *Developmental biology of agarics* (eds) D A Wood and J C Frankland (Cambridge: University Press) (in press)

Flegg P B 1968 Some recent results on the temperature requirements of the mushroom; *M G A Bull.* **218** 3–7

Fleming L V 1984 Effects of soil trenching and coring on the formation of ectomycorrhizas on birch seedlings grown around mature trees; *New Phytol.* **98** 143–153

Ford E D, Mason P A and Pelham J 1980 Spatial patterns of sporophore distribution around a young birch tree in three successive years; *Trans. Br. Mycol. Soc.* **75** 287–296

Giltrap N J 1981 Formation of primordia and immature fruiting bodies by ectomycorrhizal fungi in culture; *Trans. Br. Mycol. Soc.* **77** 204–205

Gregory P H 1982 Fairy rings; free and tethered; *Bull. Br. Mycol. Soc.* **16** 161–163
Hacskaylo E 1965 *Thelephora terrestris* and the mycorrhizae of Virginia pine; *For. Sci.* **11** 401–404
Harley J L 1971 Fungi in ecosystems; *J. Ecol.* **59** 653–668
Harper J L 1977 *Population biology of plants* (London and New York: Academic Press) 892 pp.
Harper J E and Webster J 1964 An experimental analysis of the coprophilous fungus succession; *Trans. Br. Mycol. Soc.* **47** 511–530
Hering T F 1966 The terricolous higher fungi of four Lake District woodlands; *Trans. Br. Mycol. Soc.* **49** 369–383
Hora F B 1959 Quantitative experiments on toadstool production in woods; *Trans. Br. Mycol. Soc.* **42** 1–14
Horn K 1933 Mykorrhizasopp som heksering-danner; *Friesia* **1** 38–85
Laiho O 1970 *Paxillus involutus* as a mycorrhizal symbiont of forest trees; *Acta For. Fenn.* **106** 1–72
Lamb R J 1979 Factors responsible for the distribution of mycorrhizas of *Pinus* in eastern Australia; *Aust. For. Res.* **9** 25–34
Last F T, Mason P A, Ingleby K and Fleming L V 1984a Succession of fruitbodies of sheathing mycorrhizal fungi associated with *Betula pendula; For. Ecol. Manage.* **9** 229–234
Last F T, Mason P A, Pelham J and Ingleby K 1984b Fruitbody production by sheathing mycorrhizal fungi: effects of 'host' genotype and propagating soil; *For. Ecol. Manage.* **9** 221–227
Last F T, Mason P A, Smith R, Pelham J, Bhoja Shetty K A and Mahmood Hussain A M 1981 Factors affecting the production of fruitbodies of *Amanita muscaria* in plantations of *Pinus patula*; *Proc. Indian Acad. Sci* (*Plant Sci.*) **90** 91–98
Last F T, Mason P A, Wilson J and Deacon J W 1983 Fine roots and sheathing mycorrhizas: their formation, function and dynamics; *Plant Soil* **71** 9–21
Last F T, Pelham J, Mason P A and Ingleby K 1979 Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp; *Nature* (*London*) **280** 168–169
Lewis D H 1973 Concepts in fungal nutrition and the origin of biotrophy; *Biol. Rev.* **48** 261–278
Lundeberg G 1970 Utilization of various nitrogen sources, in particular bound soil nitrogen, by mycorrhizal fungi; *Stud. For. Suec.* **79** 1–95
Manjula B 1983 A revised list of the agaricoid and boletoid basidiomycetes from India and Nepal; *Proc. Indian Acad. Sci.* (*Plant Sci.*) **92** 81–213
Mason P A, Last F T, Pelham J and Ingleby K 1982 Ecology of some fungi associated with an ageing stand of birches (*Betula pendula* and *B. pubescens*); *For. Ecol. Manage.* **4** 19–39
Mason P A, Wilson J and Last F T 1984 Mycorrhizal fungi of *Betula* spp.: factors affecting their occurrence; *Proc. R. Soc. Edinb.* **B85** 141–151
Mikola P 1965 Studies on the ectendotrophic mycorrhiza of pine; *Acta For. Fenn.* **79** 56 pp.
Miller O K 1983 Ectomycorrhizae in the agaricales and gasteromycetes; *Can. J. Bot.* **61** 909–916
Natarajan N and Raman N 1983 *South Indian Agaricales*; Cramer, Vaduz 203 pp.
Parker-Rhodes A F 1955 Fairy ring kinetics; *Trans. Br. Mycol. Soc.* **38** 59–72
Rautavaara T 1947 *Suonen sienisato* Helsinki
Richardson M J 1970 Studies of *Russula emetica* and other agarics in a Scots pine plantation; *Trans. Br. Mycol. Soc.* **55** 217–229
Rommell L G 1938 A trenching experiment in spruce forest and its bearing on problems of mycotrophy; *Sven. Bot. Tidskr.* **32** 89–99
Rommell L G 1939 Barrskogens marksvampa och deras rolli skogens liv; *Sven. Skogv For. Tidskr* **37** 238–75
Shubin V I, Ronkonen N I and Saukkonen A V 1977 The effect of fertilizers on the fructification of macromycetes on young birch trees; *Mikol. Fitopatol.* **11** 294–303
Watling R 1974 Macrofungi in the oak woods of Britain In: *The British oak: its history and natural history* (eds) M G Morris and F H Pering (Faringdon, Berks: Botanical Society of the British Isles/E W Classey)
Watling R 1984 Macrofungi of birchwoods; *Proc. R. Soc. Edinb.* **B85** 129–140
Wilkins W H and Harris G C M 1946 The ecology of the larger fungi V. An investigation into the influence of rainfall and temperature on the seasonal production of fungi in a beechwood and a pinewood; *Ann. Appl. Biol.* **33** 179–188

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 129–136.
© Printed in India.

Fungal endophytes of forage grasses

GARRY T COLE and JAMES F WHITE

Department of Botany, University of Texas, Austin, Texas 78713–7640

Abstract. Forage grasses (*e.g.*, *Lolium* spp. and *Festuca* spp.) have long been known to harbour fungi which maintain an intercellular relationship with leaf tissue of the host. These endophytic fungi are considered to be the source of toxins which accumulate in infected grasses and are the cause of physiological disorders in grazing sheep and cattle. On the other hand, these same endophytes contribute to the resistance of the host plant to certain pests. Few investigators have examined the extent to which the fungal endophyte invades different parts of the grass. Our studies have recently concentrated on the mechanism by which the endophytes of *F. arundinacea* and *F. versuta* invade the embryo and thus establish residence in successive host generations.

Keywords. Fungi; endophytes; *Festuca*; *Lolium*; *Acremonium*.

Certain species of ryegrass (*i.e.*, *Lolium* spp.) and fescue (*i.e.*, *Festuca* spp.) in the United States and New Zealand have long been recognized as hosts of fungi which reside and perennate within their leaves and seeds (Freeman 1904; McLennan 1920; Sampson 1935, 1937; Neill 1940, 1941). The fungi involved are largely members of *Acremonium* Link (Morgan-Jones and Gams 1982), although other genera of endophytic fungi have also been suggested to occur in forage grasses in New Zealand (Latch *et al* 1984). The relationship between the fungus and its host is neither that of a typical parasite nor symbiont. Considerable evidence is now available, derived mainly from studies of perennial ryegrass (*L. perenne* L.) and tall fescue (*F. arundinacea* Schreb.), that the presence of the endophyte is associated with toxic syndromes in grazing animals (Yates 1962; Porter *et al* 1975; Latch and Christensen 1982; Fletcher and Harvey 1981; Fletcher 1982; Ahmed *et al* 1982; Schmidt *et al* 1982; Harvey 1983). The presence of potent alkaloid compounds in perennial ryegrass (Aasen *et al* 1969) and tall fescue (Yates 1962) have been implicated in ryegrass staggers and fescue toxicosis of sheep and cattle, respectively. Particular attention has been directed towards, the lolitrems, tremorgenic neurotoxins obtained from *L. perenne* (Gallagher *et al* 1984), and halostachine, an alkaloid [d,l-α-(methylaminomethyl) benzyl alcohol] isolated from *F. arundinacea* which demonstrates vasoconstrictor potential in cattle (Davis *et al* 1983). Additional investigations of these and other endophyte-containing plants have indicated that the fungus may contribute to the resistance of its host to certain viral infections (Salehussaman and Wilkins 1983) as well as root invasion by fungal and insect pathogens (Prestidge *et al* 1982; Funk *et al* 1983; Barker *et al* 1983; Mortimer and di Menna 1983; White and Cole 1985b). The endophyte may also be associated with production of secondary compounds which are released from the host plant and inhibit growth and development of competitive grasses (Peters and Mohammezan 1981), in addition to exhibiting other allelopathic properties. A paradoxical situation has arisen as a result of these studies. The possibility that the endophyte is the source of compounds causing toxic syndromes in commercially valuable livestock is a good reason to develop methods to control or eradicate the

fungus (Harvey *et al* 1982; Latch 1983; Siegel *et al* 1984; Williams *et al* 1984). On the other hand, forage grasses may be provided significant benefits by the presence of the endophyte, which may translate into economically sound reasons for retaining the endophyte-plant relationship. Whether the fungus is at all detrimental to the grass is still open to question. Clay (1984) has pointed out that the distinction between a mutualistic and parasitic relationship between grass and fungus "depends on the relative fitness of infected plants compared to uninfected plants. If the fitness of infected plants is greater than uninfected plants, mutations enhancing the association would be selected for; if lower, mutations reducing the association would be selected for". In his study of the relationship between the endophytic fungus, *Atkinsoniella hypoxylon* (Peck) Diehl., and the grass, *Danthonia spicata* (L.) Beauv., it was found that infected plants produce fewer flowers but have greater survival and growth rates. The author concluded that infection decreases short term fitness, but long term survival may eventually outweigh short term losses in fecundity. It is not known if the endophytes of forage grasses have a similar effect on the fecundity of their host.

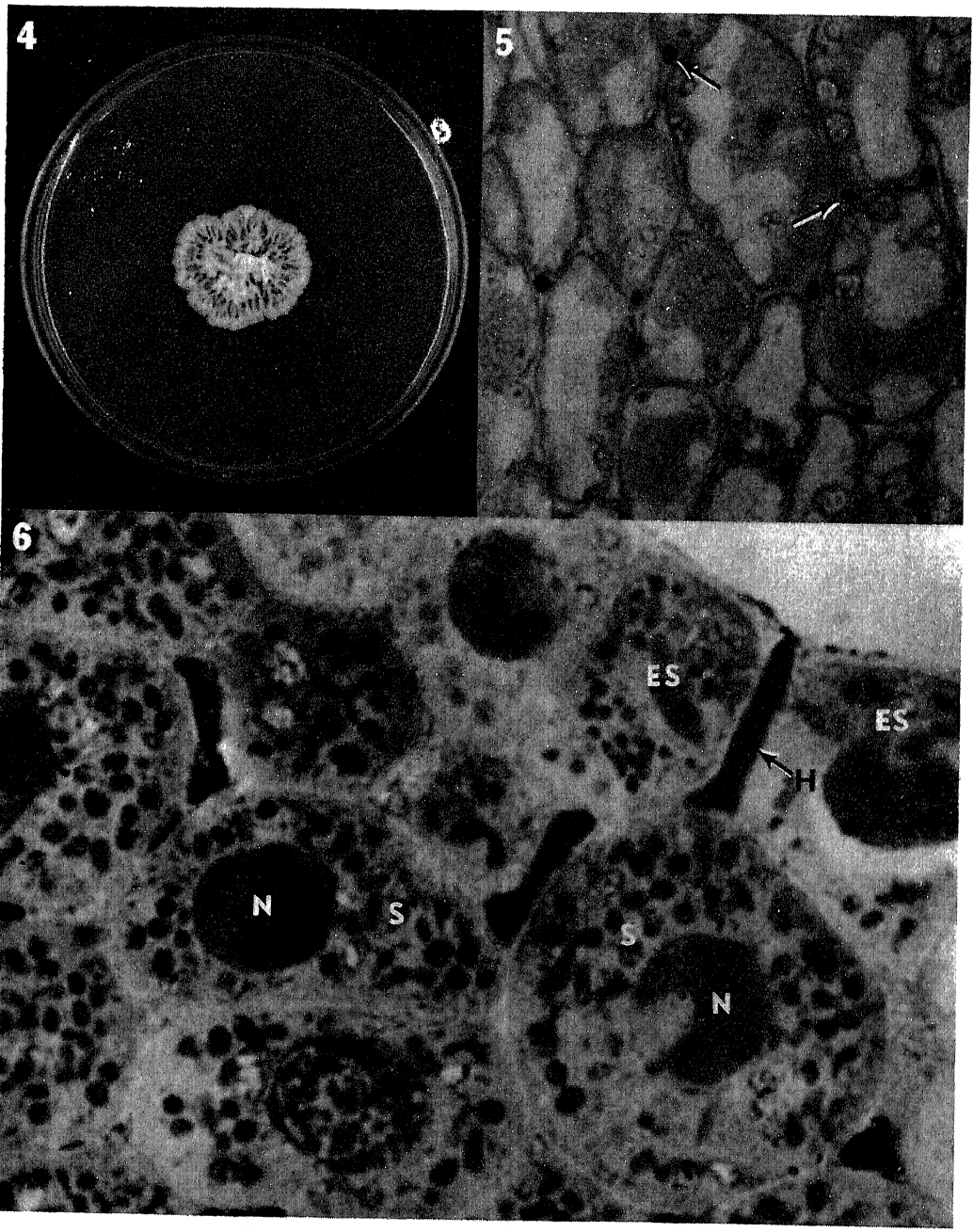
The leaf and stem tissues of *Lolium* and *Festuca* harbour extensive intercellular hyphae which can be observed under the light microscope by simply preparing mesophyll tissue squashes (figure 1) or pith scrapings (figure 2; White and Cole 1985a). The fungal hyphae are well stained with aniline blue and typically appear somewhat convoluted in the intercellular spaces of mesophyll tissue. The endophyte in the leaf is restricted to the ground parenchyma and is not found in the vascular tissue. Cryofractured and freeze-dried preparations of mesocotyl tissue of *L. perenne* (figure 3; Cole 1986) show that the hyphal wall is apparently fused to the wall of one or more of the adjacent host cells. Thin sections demonstrate this same interrelationship (Fineran *et al* 1983), but also reveal a distinctly fibrous, outer hyphal wall layer in contact with the wall of the mesophyll cell. Such surface interaction of endophyte and host, as well as the apparent tissue specificity of intercellular hyphae, suggest that a recognition phenomenon may exist between the forage grass and fungus. Cytochemical investigations are required to explore this possibility.

Homogenates of surface-sterilized seeds and leaves of *L. perenne*, *L. temulentum* L., *F. arundinacea* and *F. versuta* Beal., using 50 % Clorox (*ca.* 2·5 % NaOCl) and several water washes (Clark *et al* 1983), have yielded axenic cultures of endophytic fungi grown on potato dextrose and malt extract agar (Difco). The colonies are typically slow growing, reaching about 1–2 cm after 7 weeks at 20° C on potato dextrose agar. The isolated endophyte of *F. versuta*, unlike that of *L. perenne* and *F. arundinacea*, is sterile and its taxonomic status has not been established (figure 4). Its relationship with tissues of *F. versuta*, however, is remarkably similar to that of the endophyte of *L. temulentum* which was described in detail more than 80 years ago by Freeman (1904). Intercellular hyphae of the endophyte of both *F. arundinacea* (figure 5) and *F. versuta* are abundant in the ovary wall and throughout the nucellar tissue of the ovule

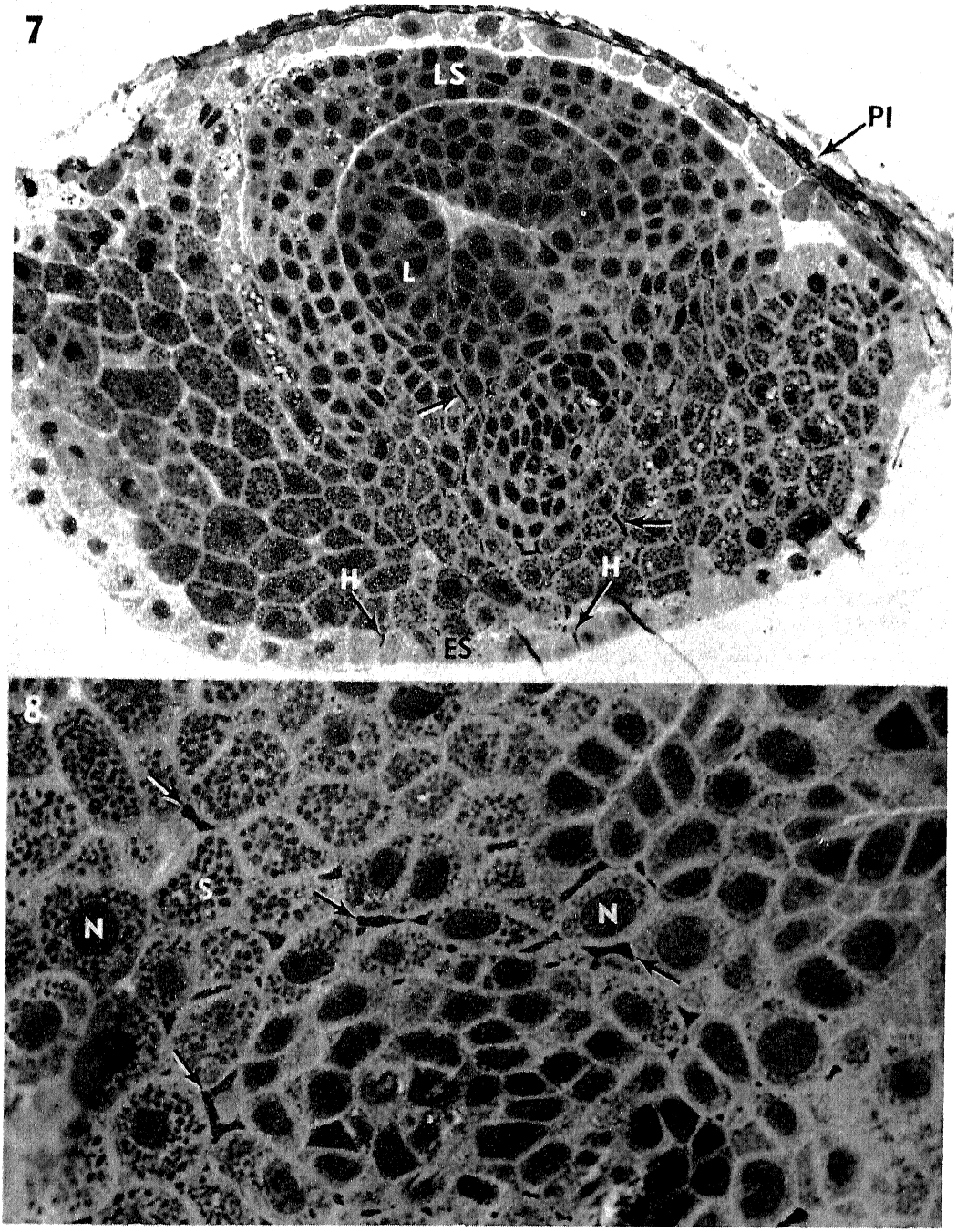
---

Figures 1–3. Fresh squash preparation of mesophyll tissue of *L. perenne* (figure 1 × 700) showing intercellular location of convoluted, septate hyphae of endophyte. Morphologically similar hyphae are found in pith scrapings of *F. arundinacea* (figure 2 × 1700). Preparations stained with aniline blue. Arrows in figure 2 locate cross walls of hypha. (HC, host cell.) **3.** Cryofractured and freeze-dried preparation of mesocotyl tissue of *L. perenne* showing intercellular, endophytic hypha (H). The wall of the hypha is typically juxtaposed to the wall of one of the host cells within the intercellular space. (× 2600).

1
HC
2
3
H



Figures 4–6. **4.** Colony of the fungal endophyte of *F. versuta* after incubation for 7 weeks on potato dextrose agar at 20°C. **5.** Section of plastic-embedded tissue of *F. arundinacea* showing the ovary wall with multiple, intercellular hyphae (arrows). Section stained with aniline blue. (× 400). **6.** Section through the outer layer of scutellum of an excised embryo of *F. versuta* showing endophytic hypha (H) penetrating between epidermal cells. Note intercellular location of other hyphal segments. (ES, epidermal cells of scutellum; N, nucleus; S, scutellum. × 500).



Figures 7,8. **7.** Section through excised embryo of *F. versuta* showing endophytic hyphae (arrows) near base of primary leaf (L). Note hyphae (H) associated with the epidermal layer of the scutellum (ES). This region is considered to be the site of ingress of the endophyte into the embryo (Freeman 1904; c.f. figure 6). (LS, leaf sheath; **PI**, fused pericarp and integuments. × 75.) **8.** Higher magnification of region at base of primary leaf of embryo (c.f. figure 7) showing multiple, intercellular hyphae (arrows) (N, nucleus; S, scutellum. × 200).

at about the time of fertilization. This same observation has been reported for the ovary of perennial ryegrass (Lloyd 1959). The integuments surrounding the young ovule of *F. versuta* are free of hyphae and the endophyte appears to enter the nucellus by way of the funiculus (White and Cole 1986). Hyphae are also absent from embryonic tissue during early development. Invasion of the embryo of *F. versuta* and *F. arundinacea* occurs by a mechanism which is comparable to that reported by Freeman (1904). The endophyte enters the embryonic tissue by penetration between the epidermal cells of the scutellum (figure 6). The hyphae subsequently proliferate within the scutellum and extend into the primary leaf tissue (figures 7,8). The fungus maintains an intercellular relationship with the embryonic tissue and no evidence of necrosis during this relatively vulnerable reproductive stage is evident. The endophyte and host have apparently achieved a compatible interrelationship and the fungus is assured survival by already establishing residence in the next generation.

The number of species of forage grasses which harbor endophytes is probably much greater than recognized at present. In the case of *Festuca*, endophytic fungi have been found in *F. arizonica* Vasey, *F. eastwoodea* Piper, *F. elatior* L., *F. ligulata* Swallen, *F. obtusa* Spreng., *F. pacifica* Piper, *F. paradoxa* Desv., *F. scabrella* Torr., and *F. subulata* Trin., in addition to *F. arundinacea* and *F. versuta* discussed above (White and Cole 1985a). Nothing is known of the fungal-host interrelationships in the majority of these species, nor is there any information on whether infected fescue grasses other than *F. arundinacea* are toxic to grazing animals. A concerted effort is necessary on an international scale to further document this unusual biological phenomenon and evaluate the positive and negative aspects of the presence of endophytic fungi in forage grasses.

Acknowledgements

The senior author is grateful to the University of Canterbury, Christchurch, New Zealand for receipt of an Erskine Fellowship, and the opportunity to discuss aspects of this paper with many of the endophyte researchers in that country. In particular, the fruitful discussions with Drs I C Harvey and M Phillipson concerning their extensive investigations of forage grass endophytes were most helpful and encouraging.

References

Aasen A J, Culvenor C C J, Finnie E P, Kellock A W and Smith L W 1969 Alkaloids as a possible cause of ryegrass staggers in grazing livestock; *Aust. J. Agric. Res.* **20** 71–86

Ahmed A, Nelson T S, Daniels L B, Piper E L and Beasley J N 1982 Fescue toxicosis: progress toward identification of its cause; *Arkansas Farm Res.* **32** 7

Barker G M, Pottinger R P and Addison P J 1983 Effect of tall fescue and ryegrass endophytes on Argentine stem weevils; *Proc. N.Z. Weed Pest Conf.* **36** 216–219

Clark E M, White J F and Patterson R M 1983 Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus; *J. Microbiol. Methods* **1** 149–155

Clay K 1984 The effect of the fungus *Atkinsonella hypoxylon* (Clavicipitaceae) on the reproductive system and demography of the grass *Danthonia spicata*; *New Phytol.* **98** 165–175

Cole G T 1986 Preparation of microfungi for scanning electron microscopy. In: *Ultrastructure techniques for microorganisms* eds H C Aldrich and W J Todd (New York: Plenum Press)

Davis C B, Camp B J and Reed J C 1983 The vasoactive potential of halostachine, an alkaloid of tall fescue (*Festuca arundinacea* Schreb.) in cattle; *Vet. Hum. Toxicol.* **25** 408–411

Fineran B A, Harvey I C and Ingerfeld M 1983 Unusual crystalloids and aggregates of tubules in the *Lolium* endophyte of ryegrass leaf sheaths; *Protoplasma* **117** 17–23

Fletcher L R 1982 Observations of ryegrass staggers in weaned lambs grazing different ryegrass pastures; *N. Z. J. Exp. Agric.* **10** 203–207

Fletcher L R and Harvey I C 1981 An association of a *Lolium* endophyte with ryegrass staggers; *N. Z. Vet. J.* **29** 185–186

Freeman E M 1904 I. The seed-fungus of *Lolium temulentum* L., the darnel; *Philos. Trans.* **196** 1–27

Funk C R, Halisky P M, Johnson M C, Siegel M R, Stewart A V, Ahmad S, Hurley R H and Harvey I C 1983 An endophytic fungus and resistance to sod webworms: association in *Lolium perenne* L; *Bio. Technol.* **1** 189–191

Gallagher R T, Hawkes A D, Steyn P S and Vleggaar R 1984 Tremorgenic neurotoxins from perennial ryegrass causing ryegrass staggers disorder of livestock: structure elucidation of lolitrem B; *J. Chem. Soc., Chem. Commun.* No. 9 614–616

Harvey I C 1983 The *Lolium* endophyte: return from anonymity; *Ann. Proc. N. Z. Grasslands Assoc.* **44** 234–236

Harvey I C, Fletcher L R and Emms L M 1982 Effects of several fungicides on the *Lolium* endophyte in ryegrass plants, seeds, and in culture; *N. Z. J. Argic. Res.* **25** 601–606

Latch G C M 1983 Incidence of endophytes in seed lines and their control with fungicides; *Proc. N. Z. Grasslands Assoc.* **44** 251–253

Latch G C M and Christensen M J 1982 Ryegrass endophyte, incidence and control; *N. Z. J. Agric. Res.* **25** 443–448

Latch G C M, Christensen M J and Samuels G J 1984 Five endophytes of *Lolium* and *Festuca* in New Zealand; *Mycotaxon* **20** 535–550

Lloyd A B 1959 The endophytic fungus of perennial ryegrass; *N. Z. J. Agric. Res.* **2** 1187–1194

McLennan E 1920 The endophytic fungus of *Lolium*. Part I; *Proc. R. Soc. Victoria* **32** 252–301

Morgan-Jones G and Gams W 1982 Notes on Hyphomycetes, XLI. An endophyte of *Festuca arundinacea* and the anamorph of *Epichloe typhina*, new taxa in one of two new sections of *Acremonium*; *Mycotaxon* **15** 311–318

Mortimer P H and di Menna M 1983 Ryegrass staggers: further substantiation of a *Lolium* endophyte aetiology and the discovery of weevil resistance of ryegrass pastures infected with *Lolium* endophyte; *Proc. N. Z. Grasslands Assoc.* **44** 240–243

Neill J C 1940 The endophyte of ryegrass (*Lolium perenne*); *N. Z. J. Sci. and Technol.* **A21** 280–291

Neill J C 1941 The endophytes of *Lolium* and *Festuca*; *N. Z. J. Sci. and Technol.* **A23** 185–193

Peters E J and Mohammezan A H B 1981 Allelophatic effects of tall fescue (*Festuca arundinacea*) genotypes; *Agron. J.* **73** 56–58

Porter J K, Bacon C W, Robbins J D and Higman H C 1975 A field indicator in plants associated with ergot-type toxicities in cattle; *J. Agric. Food Chem.* **23** 771–775

Prestidge R A, Pottinger R P and Barker G M 1982 An association of *Lolium* endophyte with ryegrass resistance to Argentine stem weevil; *Proc. N. Z. Weed Pest Control. Conf.* **35** 199–222

Salehussaman M and Wilkins P W 1983 Inhibitory activity in *Lolium perenne* associated with resistance to infection by ryegrass mosaic virus; *Physiol. Plant Pathol.* **22** 199–207

Sampson K 1935 The presence and absence of an endophytic fungus in *Lolium temulentum* and *L. perenne*; *Trans. Br. Mycol. Soc.* **19** 337–343

Sampson K 1937 Further observations on the systemic infection of *Lolium*; *Trans. Br. Mycol. Soc.* **21** 84–97

Schmidt S P, Hoveland C S, Clark E M, Davis N D, Smith L A, Grimes H W and Helliman J L 1982 Association of an endophytic fungus with fescue toxicity in steers fed Kentucky 31 tall fescue seed or hay; *J. Anim. Sci.* **5** 1259–1263

Siegel M R, Varney D R, Johnson M C, Nesmith W C, Buckner R C, Bush L P, Burns P B and Hardison J R 1984 A fungal endophyte of tall fescue: evaluation of control methods; *Phytopathology* **74** 937–941

White J F Jr and Cole G T 1985a Endophyte-host associations in forage grasses. I. Distribution of fungal endophytes in *Lolium* and *Festuca*; *Mycologia* **77** 323–327

White J F Jr and Cole G T 1985b Endophyte-host associations in forage grasses. III. *In vitro* inhibition of fungi by *Acremonium coenophialum; Mycologia* **77** (in press)

White J F Jr and Cole G T 1986 Endophyte-host associations. IV. The endophyte of *Festuca versuta; Mycologia* (in press)

Williams M J, Backman P A, Clark E M and White J F 1984 Seed treatment for control of the tall fescue endophyte *Acremonium coenophialum*; *Plant Dis.* **68** 49–52

Yates S G 1962 Toxicity of tall fescue forage: a review; *Econ. Bot.* **16** 295–303

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 137–148.
© Printed in India.

Ambrosia beetles and their associated fungi: research trends and techniques

L R BATRA

Mycology Laboratory, Plant Protection Institute, U. S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, Maryland 20705, USA

Abstract. General characteristics of ambrosia fungus-beetle mutualism are described and recent trends for innovative research briefly indicated. Techniques to locate, collect, transport, maintain, isolate, and study fungi and beetles in the field and in vitro are reviewed in detail. A synthetic basal medium for growing ambrosia fungi is described; this should enable researchers to investigate nutrition and enzymology of both the symbionts.

Keywords. Ambrosia beetles; ambrosia fungi; symbiosis; mutualism.

1. Introduction

I express my appreciation to President Prof. S Ramaseshan and the Editorial Board of the Proceedings of the Indian Academy of Sciences (Plant Sciences) for inviting me to contribute towards this Special Issue to commemorate the 60th birthday of my good friend Prof. C V Subramanian. After many months of contemplation I have chosen to write on a subject that should please "CV", an inspiring teacher and trainer of many generations of students. Peers more familiar than I undoubtedly shall comment on his diverse contributions, and the recognition and respect he has earned from academia, scientific organizations worldwide, and the Government of India.

A considerable portion of my research life has been devoted to symbiosis (mutualism) between fungi and insects. Apart from academic interest, most of these symbioses are of great economic concern, *e.g.*, fungus-growing termites (Macrotermitidae: Isoptera) of the old world, attine ants (Hymenoptera) of the new world tropics, and diverse fungus-growing beetles (Xyleborini, Platypodideae, and Lymexylonidae: Coleoptera). Here I shall discuss only the fungus-growing beetles and their mutualistic fungi. This research is tedious and of multidisciplinary nature. It requires commitment from interested professionals throughout its progress, particularly competent judgement at the time of collection of research material, the establishment of insect colonies, and microbial cultures. Periodic purity or "sterility" tests are pre-requisites for working with axenic paired symbionts. Dr S W T Batra, an entomologist and animal behaviorist (U S Department of Agriculture, Beltsville), and I have profitably co-investigated the mutualism of the aforementioned taxa for the past 26 years.

The fungivorous, wood-inhabiting beetles called "ambrosia beetles" (see terminology below) live mutualistically with certain species-specific fungi. The beetles shelter the fungi in specialized pockets lined with glandular tissue during their periods of quiescence or overwintering. These pockets (mycangia) also provide nutrition for multiplication of their fungi during favourable periods of growth. The fungus

inoculum overflows or sloughs off into the beetles' tunnels during excavation or during oviposition. Mechanical injury to wood elements due to burrowing by the insect allows rapid fungus penetration. The fungus also benefits from nitrogenous wastes of the beetle as sources of nutrients. The beetle benefits because larvae in most cases are exclusively mycetophagous, and also because the fungus may weaken wood elements for several days prior to hatching of eggs, thus facilitating cell excavation or enlargement of tunnels by larvae. Subsequent succession of various non-specific wood rotting fungi through insect burrows hastens the processes of decay and deterioration and recycling of nutrients through the forest ecosystem.

Ambrosia beetles worldwide are a significant factor in the proper utilization of trees, timber, and forest products. Some species attack living trees or bushes and, along with their associated fungi, cause extensive economic harm to crops. The tea shot-hole borer, *Xyleborus fornicatus* Eichh. (symbiotic fungus: *Monacrosporium ambrosium* Gadd and Loos (1947)), attacks living tea bushes in Sri Lanka, Java, and Borneo (Kalshoven 1958) and in India. The coffee-twig borer (also called shot-hole borer), *Xylosandrus compactus* Eichh. (symbiotic fungus: *Ambrosiella xylebori* Brader (1964)), similarly causes extensive damage to living *Coffea* spp. in Africa and India (Muthappa and Venkatasubbaiah 1981). It is also prevalent in tea bushes in Japan (Kaneko 1965).

Lately the concept of mutualism has been extended to include bark beetles which harbor certain fungi (species specificity not well documented), in elytral declivities. In addition to feeding on bark, these beetles apparently also eat the fungi (Bridges 1983 and references cited therein).

Since mutualistic insect pests in general will not survive or reproduce in nature without their mycosymbionts, *i.e.* aposymbiotically, it is evident that controlling the fungus may also control the insect or vice versa. In the closing paragraph of a symposium six years ago I stated, "At present there are no practical ways to control insect pests by aposymbiosis, and any chemicals to control symbionts must not select resistant forms. It is surprising that hardly any basic research is being conducted on the role of aposymbiosis as a tool to restrain arthropod pests, within the overall strategy of integrated pest management, including control by microbial pathogens which are well reviewed . . . Aposymbiosis may debilitate our enemy, and anything that debilitates our enemy must be looked into—may it even be a chariot wheel!" (Batra 1979). In order to exploit aposymbiosis as a tool to control pests that utilize microsymbionts we must conduct fundamental research on the nutrition of the insect symbiont and on the role of the microsymbiont in its metabolism (see Kok and Norris 1973) and also conduct research on aggregating pheromones and behavioral effects of other compounds that emanate from tunnels colonized by wood-boring insects and their symbionts, *e.g.*, various pinenes and their derivatives, and even a molecularly simple compound, ethanol (Magema *et al* 1982; Lindgren and Borden 1983).

It is notable that during several trips to Asia and Europe between 1964–82 we found much interest in our work on insect-fungus symbiosis. However as in the English-speaking world, this interest was largely shown by entomologists, or persons not directly studying fungi. For example, in a recent symposium (Batra 1979), six of the nine authors who discussed insect-fungus mutualism were entomologists by training and this is apparently the case in other similar series. Since questions most often addressed to us relate to methods or techniques to collect, transport, maintain, isolate and characterize ambrosia fungi and their mutualistic beetles, I update these topics here. Unless stated otherwise, all techniques are from our publications, as cited in the

symposium mentioned above. Techniques to investigate cellulases with xylophagous insects (Chararas 1981), or proteases and cellulases of fungivorous termites and ants (Martin 1984) may be adapted in studies of beetle-fungus mutualism.

2. Terminology and recent literature

2.1 *Ambrosia beetles*

These beetles live in obligatory symbiosis with their mutualistic fungi. *Ambrosia* is the fungal mass upon which larvae, and sometimes adults, of the families Scolytidae, Platypodidae, and Lymexylonidae feed. The Scolytidae, widely distributed in temperate and tropical regions, include the important ambrosia-feeding genera *Xyleborus, Trypodendron, Gnathotrichus*, and *Anisandrus*, but many scolytid genera are not fungus feeders. The Platypodidae are mostly tropical, all are fungus eaters, and among the common ones are the genera *Platypus, Crossotarsus* and *Diapus*. Ambrosia (= ambrosial cells, ambrosial state) also refers to monilioid yeast-like vegetative hyphal fragments (= torulose hyphae constricted at regular intervals) or sprout cells. Such fragments are found in mycangia and in tunnels. They constitute the bulk of the ambrosia colonies on agar media where beetles have been feeding or are otherwise active. Ambrosial cells, under appropriate conditions, given rise to vegetative mycelium and vice versa. This phenomenon is referred to as pleomorphism. For a popular account see Batra and Batra (1967) and for technical details and a classification of ambrosia fungi see Batra (1967).

2.2 *Primary ambrosia fungi*

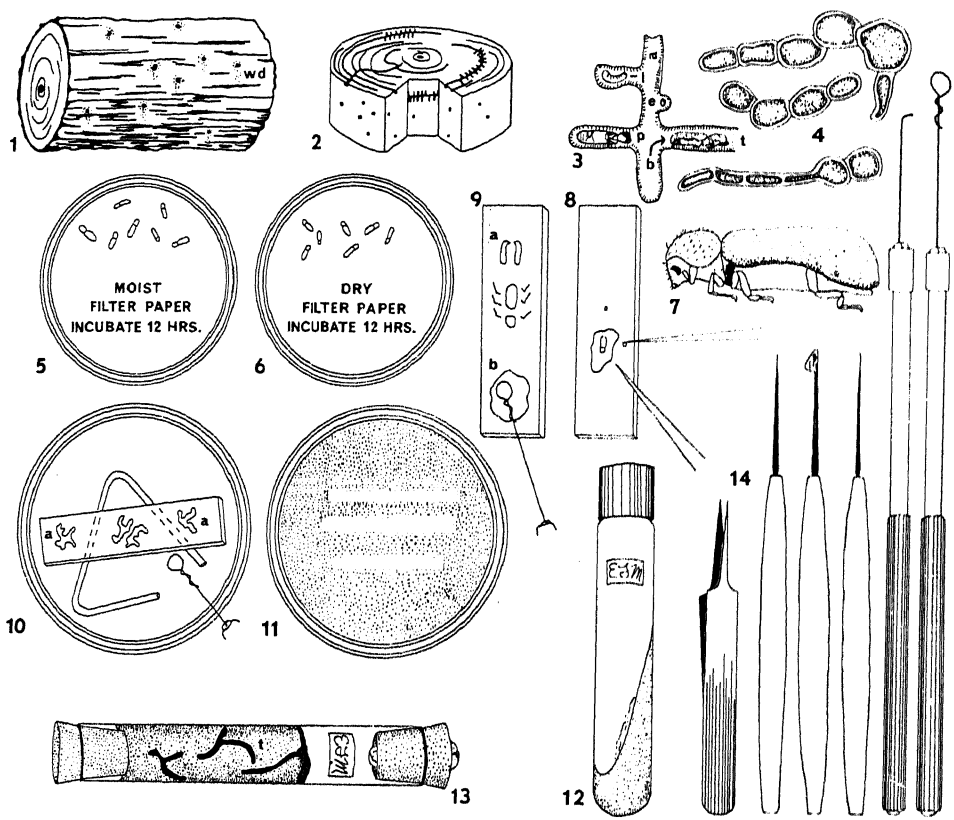
They are (i) highly species specific fungi, and their distribution ranges correspond with those of their insect symbionts; (ii) they are present in the tunnels from the beginning and are isolated regularly from the mycangium of the beetles in flight stage or when excavating tunnels; (iii) they are observed being eaten by larvae, and (iv) they are dominant and isolated from the galleries during peak growth of the brood. These obligately mutualistic fungi are extremely susceptible to drought and are generally not found outside the mycangia or the galleries of the beetles; for further details see Batra (1966). Some examples of true ambrosia fungi are *Ascoidea* Brefeld and Lindau, *Ambrosiella* von Arx and Hennebert, *Raffaelea* von Arx and Hennebert and *Phialophoropsis* Batra.

2.3 *Auxiliary ambrosia fungi*

These are (i) transitory, non-specific with respect to insect symbiont and may appear in galleries after the peak of the brood; (ii) they may or may not be present facultatively in larval cradles or in adult beetles; (iii) their habitat and distribution range are unrestricted and unrelated to that of the accidentally associated ambrosia beetles. Many such opportunistic fungi have mucilaginous spores and they are adapted for external transmission by diverse invertebrates, especially insects and mites. Many auxiliary fungi are easy to culture and have been mistaken for pure ambrosia species; (see for example table 2.1, Kok, and table 3.1, Norris, both *in* Batra 1979). The most common examples of auxiliary ambrosia fungi are *Ceratocystis* spp.

2.4 *Mycangia*

They are also called *mycetangia* and are ectodermal glandular pockets or invaginations of beetles where ambrosia fungi are stored and where they often multiply. During excavation of tunnels, the ambrosia is inoculated into the wood when it extrudes from mycangia and is dislodged. For fundamental details see Batra (1963a), Francke-Grossmann (1956, 1963, 1967); for meticulous and beautiful work with a scanning electron microscope see Nakashima (1972, 1975, 1979).



Figures 1–14. **1.** A beetle infested log, 40–50 cm long and 15–20 cm in diam, suitable for incubation in the laboratory; wood dust (wd) around tunnel entrance indicates beetle activity. **2.** Cross-section of a log exposing tunnels. **3.** A brood tunnel with an egg (*e*), a larva (*l*), a pupa (*p*), an adult (*b*), and a palisade, of ambrosia (*a*). **4.** Ambrosia cells, lower most fragment forming an elongate hyphal tip. **5, 6.** Moist and dry incubation dishes for disinfestation of beetles. **7.** An ambrosia beetle with a pleural mycangium (solid black area). **8, 9.** Microscope slides used for the dissection of mycangia. Note beetle parts separated in figure 9a; each part is further dissected in a fresh drop of bovine serum, (figure 9b). **10.** Ambrosia (*a*) on a microscope slide and being incubated in a moist petri dish. **11.** Ambrosia from palisade streaked on a layer of nutrient agar in a petri dish. **12.** A stock culture tube. **13.** A tube, with both ends plugged, used to rear beetles. **14.** Assorted dissection tools and transfer needles (figure 4 × 500, all others diagrammatic).

3. Occurrence and location of material

Locate hardwood or coniferous trees that have been weakened by drought, flooding, disease, or fire, or recently fallen timber that is moist and still filled with sap. A clean cut through a tunnel of an ambrosia beetle usually shows brownish or blackish discoloration in wood around the hole, which is clean, and has no wood dust or frass. The entrance to the tunnel system of the beetles is a small, neat circular hole only slightly larger than the diameter of the insect's body. When beetles are active inside the wood, there is usually fresh, fine wood powder outside the entrance (figure 1). This powder accumulates in the bark crevices, at the base of a tree or a log, or on the surface of infested wood if it is lying horizontally. Occasionally this powder, along with dark brownish minute pellets of larval frass, is extruded in the form of compact cylinders (0·25–3 cm long) tightly fitting in the entrance. In all cases, the beetle larvae feed on the ambrosia fungus that lines almost the entire tunnel system. Blow away the wood dust to see the mother beetle, who is often present at the tunnel entrance. Some useful tools and media are: chain saw, laboratory type band saw or a saber saw, hatchet, wood chisel, garden pruning shears (to slice open larval cradles), insect net (fine mesh), sterile vials with a damp filter paper on the bottom (to be used as inserts in insect aspirator), sterile microscope slides, moist chambers, dissection tools of carborundum steel and of fine quality (see figure 14), and laboratory media, as discussed in the text.

Some ambrosia beetles are attracted by the odour of fermented sap and can be seen flying upwind, usually at dusk. It is easy to collect them around a felled log, with a fine mesh insect net or with an aspirator containing a sterile vial with damp, but not wet, filter paper to receive beetles. As stated in an earlier paper (Batra and Batra 1967), "They are similarly attracted by the yeasty smell of beer and beer drinkers, and it is also convenient to collect them at a beer picnic! Some kinds bore into beer and wine kegs, which is why in Europe they are called 'beer beetles' ".

4. Collection of material

Wash under running water and gently scrub beetle-infested logs (usually 40–50 cm long and 15–20 cm in diam) to remove loose dirt, mites, and eggs of undesirable biota such as spiders and other predators (figure 1). The logs should then be wiped with cheesecloth dipped in ethyl alcohol and any excess flamed away. Repeat disinfestation three to five times. Smear the cut ends of the logs with petroleum jelly, or preferably, with paraffin wax to prevent dehydration and infestation by undesirable saprobes. We routinely incubated logs at room temperature and at relative humidity 80–90 % in 40- or 80-liter glass terraria supported on dishes containing mineral oil as a barrier to avoid mite infestation of our fungus culture stock. Logs can also be stored for one to two months in cardbord boxes lined with a sheet of polyethylene, but such an arrangement is inconvenient for periodic observations.

Saw off a cross-section of a log (figure 2), subsequently slice it aseptically with a hatchet or a wood chisel, expose brood tunnels (figure 3), and hold these wood chips in a sterile petri dish for observations.

Confirm taxonomic identity of the ambrosia beetle-fungus system to ascertain that one is dealing with a true ambrosia fungus and not with an auxiliary fungus or secondary contaminants and proceed as follows: (a) observe whether or not larvae are

feeding on ambrosia in tunnels or cells (cradles); and (b) determine presence of ambrosial cells microscopically before plating them (figure 4).

5. Isolation and maintenance of fungi taken from beetles

For higher rates of successful isolations, make isolations as soon as adults are collected and use beetles in the following order of priority: (a) those just beginning to vigorously tunnel after a period of quiescence or overwintering—these would include males where only this sex has mycangia and initiates tunnel excavation, *e.g. Gnathotrichus*, or where both sexes have mycangia and both may excavate tunnels, singly or as a pair, *e.g. Platypus* and *Diapus*; (b) flying insects or those active in tending brood, including cleaning of tunnel system (figure 3b); (c) young adults about to migrate, or when beginning to enlarge parent tunnel system to begin another generation; (d) adults about to overwinter in litter. The lowest rate of successful isolations from (d) is probably associated with the large quantity of fat that is released from various tissues during dissection and may prevent or retard growth of inoculum in mycangia. Likewise, old, worn-out females usually do not yield ambrosia fungus but rather yield contaminants. Since quiescent inocula of foreign fungi are usually present, even in well-tended tunnels, ambrosia-smeared larvae and pupae are not as suitable for isolations as adults. The best success occurs when mycangia of overwintered beetles could be located, excised intact, further dissected, and the ambrosia streaked on microscope slides (see below). For disinfestation prior to isolation of ambrosia fungi, allow beetles to crawl on sterile moist filter paper (avoid excessive free water) in a petri dish for 2 hr. Transfer beetles to another moist petri dish for 12 hr (figure 5); then incubate them in a dry petri dish (figure 6) for the same length of time. This alternate incubation in moist and dry chambers should be repeated twice or thrice to reduce any contamination present on the exterior of the beetles (Francke-Grossmann 1956). We obtained fungus-free adults from pupae incubated in dry sterile petri dishes and Norris and Baker (1969) submerged pupae of *Xyleborus ferrugineus* from laboratory stock cultures in 0·1 % $HgCl_2$ for 4 min, rinsed in sterile distilled water, and then aseptically placed them on potato dextrose agar to obtain fungus-free adults; see Weber and McPherson (1983) for a recent laboratory study on *Xylosandrus germanus*.

Aseptically place each beetle (figure 7) in a drop of bovine serum or sterile skimmed milk on a microscope slide (figure 8). Separate elytra, head, pronotum, mesonotum, metanotum, and abdomen (figure 9a) and crush each part in a new drop of the serum (figure 9b) with the aid of dissection needles or forceps. Allow them to remain in the serum for 15–30 min to obtain osmotic equilibrium.

Examine each crushed part and surrounding mountant for the presence of ambrosia (figure 4) and aseptically streak it on a microscope slide bearing a 0·5 mm thick layer of yeast-extract-malt-extract agar (figure 10). Using a compound microscope mark well separated ambrosia cells (figure 10a) or propagules with a sterile needle or by other suitable means, incubate the slide in a moist chamber, and observe daily until fungi begin to grow. When plated from mycangia, most of the 60 species of primary ambrosia fungi known to us were very slow growing and often 8–10 days elapsed before new cells could be found on agar. We do not recommend use of antibiotics to suppress bacteria in slide cultures for they further retard new growth from the ambrosia.

Aseptically scrape and transfer ambrosia from the fungus palisade in tunnels or cradles (figure 3a) of a vigorous brood directly onto acidified potato dextrose agar plates, or on malt agar with 30 ppm streptomycin sulphate and 10 units per ml of penicillin to avoid bacterial contamination (figure 11). Occasionally ambrosia propagules from mycangia or gallery palisade do not grow further on agar media but when incubated in malt-peptone broth with antibiotics they form additional ambrosia cells, mycelium, or both. In such cases, obtain pure stock from broth cultures streaked onto suitable agar medium. On rare occasions one can conveniently obtain ambrosia growth from wood transverse sections passing adjacent to tunnels and incubated in a moist chamber. Transfer this ambrosia directly onto suitable agar media *e.g.* (see figure 19 *in* Batra 1967).

For stock cultures, transfer ambrosia growth or tips of hyphae from ambrosia propagules onto partial strength yeast-extract-malt-extract agar (3 g/l yeast extract, 10 g/l malt extract, 20 g/l agar), malt agar (10 g/l malt extract, 20 g/l agar), or Difco potato dextrose agar. Store fungi at 5°C and transfer every 3–6 months. Most ambrosia fungi do not survive freeze drying but mineral oil may be used to store them for up to two years.

For characterization and nutritional studies of filamentous ambrosia fungi we used a synthetic medium with the following composition (Batra 1959, 1963b); for yeasts and related species see Lodder (1970); for other groups consult a text on fungal physiology and nutrition. Our medium contains NH_4NO_3 or an equivalent amount of asparagin, 3 g; KH_2PO_4, 2 g; $MgSO_4 \cdot 7\ H_2O$, 0·75 g; $FeSO_4$, 0·002 g; dextrose, 10 g; Difco agar, 25 g; distilled water, 1 liter. A vitamin mixture was added just before dispensing it into plates in autoclaved, cooled medium to yield the following final concentration (in mg/l): ascorbic acid, 0·10; biotin, 0·005; inositol, 5·00; pyridoxine hydrochloride, 0·10; riboflavin, 0·10; and thiamin (aneurine) hydrochloride, 0·10.

6. In vitro culture of ambrosia beetles

From routinely maintained logs (see §4) or beetles caught in the air, in vitro colonies can be attempted for observations on their behaviour and bionomics under controlled environments. The simplest method is to incubate pieces of natural debarked host wood containing the brood in a moist petri dish. This is useful only for a week or so because contaminants soon take over. Nobuchi (1972) reared *Xylosandrus germanus* and *Xyleborus compactus* in test tubes containing steam sterilized tea twigs, roots, and mulberry stems. Singh (1977) describes many diets to rear insects and some of them may be adaptable for in vitro culture of the ambrosia beetles.

Aseptic colonies of beetles can be reared from eggs and fungus-free adults on the following medium in wide mouth, 50–100 ml bottles, flasks, or most conveniently in test tubes (150 × 20 mm) open at both ends which have no. 2 rubber stoppers, one with a hole plugged with cotton (figure 13): Mix *Populus* saw dust (it may be substituted with that of infected plant), oven dried, 100 g; potato starch, 10 g; Difco yeast extract, 3 g; sucrose, 10 g; streptomycin sulphate, 10 mg; penicillin, 1250 units; distilled water, 125 ml. Lightly pack tubes smeared with glycerin, to a height of about 5 cm, plug and autoclave for 15 min at 15 psi/2·27 kg/cm^2. Inoculate tubes with the specific ambrosia fungus 2–3 days prior to introducing the desired number of fungus-free adults or disinfested eggs (see §5) and incubate in the dark at the desired temperature, usually at

about 22–26°C. Beetles so far cultured began burrowing against the tube wall (figure 13, *t*), thus providing desirable conditions to observe their behaviour. When the first eggs are visible, the entire medium, along with the brood, may be ejected into a sterile petri dish or another suitable observation chamber. We routinely maintained colonies of *Xyleborus saxseni, X. politus* (figures 15, 16), and *Monarthrum fasciatum* (figure 18) this way for many generations.

For techniques to study nitrogen requirements of fungi and beetles, consult French and Roeper (1972, 1973), who meticulously investigated many aspects of obligate mutualism between *Xyleborus dispar* and *Ambrosiella hartigii* Batra. For similar studies on sterols consult Kok and Norris (1973). The latter authors studied the nutritional adequacy of various sterols or related compounds for the growth, development, and reproduction of fungus-free *Xyleborus ferrugineus*. To rear beetles for routine work Norris and his group use the following medium (Norris and Baker 1969): mix the following ingredients (in g) in a blender for 1 min: sucrose, 20; casein, vitamin free*, 20; Wesson's salts*, 5; sorbic acid, 0·4; streptomycin, "20 % wettable", 0·35; Difco agar, 10; cholesterol, 0·4; vitamin solution**, 20 ml; and distilled water, 230 ml. Add 15 g pure powdered cellulose* and 4 g fibrous cellulose (Johnson and Johnson) and hand stir all ingredients. Autoclave the diet for 20 min at 2·27 kg/cm^2, aseptically dispense in sterile tubes (150 × 20 mm) to about a height of 5 cm level, and allow "to dry at room temperature for 1 day", roughen diet surface with a sterile needle and introduce 1 beetle per tube. One day later inoculate each tube with a small plug of fungus from the stock culture and incubate in dark at desired temperature, usually between 25–30°C.

An addition of 2·5 ml of 95 % ethanol in the diet stimulated tunnelling by the beetles; this practice gave more progeny than the medium without ethanol (Norris and Baker 1969).

French and Roeper (1972) medium for the maintenance of beetle colonies is as follows: for one liter of medium, mix dry: glucose, 18·8 g; L-asparagine, 1 g; Wesson's Salt Mixture, 0·5 g; alpha cellulose, 190 g; melt 45 g of Bacto agar in 500 ml of distilled water, then stir into the medium, with a further 500 ml of hot distilled water. Dispense into tubes 150 × 20 mm about 10 ml of this viscous medium through a squeeze-type dispenser such as a cake decorating device sold in grocery stores, plug them with cotton, autoclave for 20 min at 2·27 kg/cm^2, and cool. Two to five days prior to the introduction of the female parent inoculate tubes with the specific ambrosia fungus from the stock culture, preferably an isolate from the mycangia. French and Roeper (1972, 1973) usually disinfested beetles by alternate incubation in wet and dry petri dishes (see §5), transferred them with a sterile vacuum tweezer system and incubated the cultures in the dark at about 20–24°C.

7. Histochemistry of mycangia

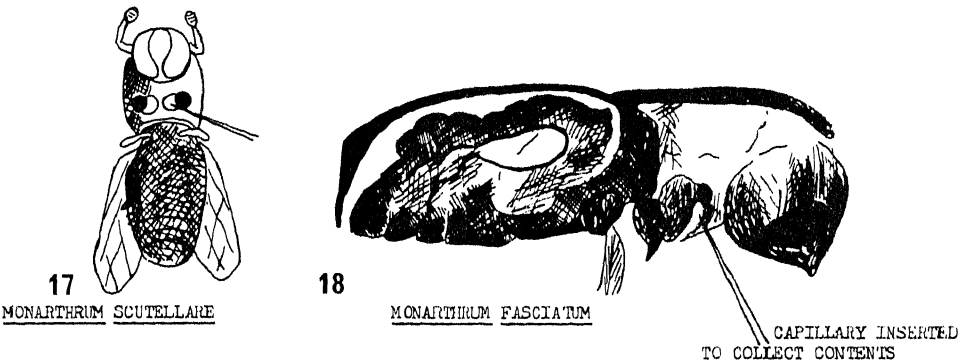
Ambrosia beetles and their mycangial secretions apparently exert significant influence on morphogenesis and biology of the ambrosia fungi. Conidia and other spore forms

* Source: ICN Nutritional Bichemical Co., 36301 Miles Road, Cleveland, Oho 44128. Mention of a trade name or manufacturer does not constitute an endorsement of the product by the US Department of Agriculture over other products not mentioned.

** A solution containing the following quantities, in g, of each vitamin per litre: choline chloride, 10·3; inositol, 4·6; calcium pantothenate, 0·85; niacin, 0·33; p-aminobenzoic acid, 0·155; riboflavin, 0·048; pyridoxine HCl, 0·037; thiamine HCl, 0·028; folic acid, 0·011; biotin, 0·008; and cobalamine B12, 0·002.

Figure 15. For caption see p. 146

Figures 15, 16. 15(about × 2). A core of wood dust medium with tunnels, eggs and adults of *Xyleborus politus*; arrows point to layer of ambrosia and eggs. **16**(× 18). Eggs and ambrosia



Figures 17, 18. Precoxal mycangia of *Monarthrum* spp., diagrammatic.

of these fungi are transformed into the ambrosia stage within the mycangia (Batra 1967). Mycangial secretions contain several oils and proteins. They may serve as nutrients as well as preservatives for the inoculum, much like mineral oil used to preserve fungal cultures. For histological techniques to study mycangia see Schneider and Rudinsky (1969a, b) who investigated seasonal changes in the glands surrounding mycangia and their anatomy but much additional work is desirable in this direction.

Biochemistry of mycangial contents is unknown although some beetles with relatively robust and easily accessible mycangia offer promise, *e.g. Monarthrum, Typodendron*, and *Gnathotrichus*. In *Monarthrum* during early spring we collected mycangial contents with minute glass capillary tubes (figures 17, 18) and found fat (Sudan III test), protein, and amino acids with thin layer chromatographic techniques. By incubating capillary tubes in moist petri dishes we could also follow the development of mycangial inoculum.

Acknowledgement

I thank Marla Hunt for assistance in the preparation of this manuscript and Elizabeth Compton Montez and S W T Batra for preparing the figures.

References

Batra L R 1959 A comparative morphological and physiological study of the species of *Dipodascus; Mycologia* **51** 329–355

Batra L R 1963a Ecology of ambrosia fungi and their dissemination by beetles; *Trans. Kansas Acad. Sci.* **66** 213–236

Batra L R 1963b Habitat and nutrition of *Dipodascus* and *Cephaloascus; Mycologia* **55** 508–520

Batra L R 1966 Ambrosia fungi: extent of specificity to ambrosia beetles; *Science* **153** 193–195

Batra L R 1967 Ambrosia fungi: a taxonomic revision, and nutritional studies of some species; *Mycologia* **59** 976–1017

Batra L R 1979 (ed.) *Insect-fungus symbiosis: nutrition, mutualism and commensalism* (Montclair, New Jersey: Allenheld, Osmun and Co.) pp. 276

Batra S W T and Batra L R 1967 The fungus gardens of insects; *Sci. Am.* **217** 112–120

Brader L 1964 Etude de la relation entre le scolyte des rameaux du caféier, *Xyleborus compactus* Eichh. (*X. morstatti* Hag.), et sa plante-hote; *Meded. Landbouwhogesch.* **64**(7) 1–109

Bridges J R 1983 Mycangial fungi of *Dendroctonus frontalis* (Coleoptera: Scolytideae) and their relationship to beetle population trends; *Environ. Entomol.* **12** 858–861

Chararas C 1981 Origin and specificity of certain cellulases and hemicellulases in different xylophage insects; *C. R. Seances Acad. Sci. Ser. III Sci. Vie* **293** 211–214

Francke-Grosmann H 1956 Haudrüsen als Träger der Pilzsymbiose bei Ambrosiakäfern; *Z. Morphol. U. Ökol. Tiere* **45** 275–308

Francke-Grosmann H 1963 Some new aspects in forest entomology; *Ann. Rev. Entomol.* **8** 415–438

Francke-Grosmann H 1967 Ectosymbiosis in wood-inhabiting insects. In *Symbiosis* (ed.) S M Henry (New York: Academic Press) Vol. 2 pp. 141–205

French J R J and Roeper R A 1972 In vitro culture of the ambrosia beetle *Xyleborus dispar* (Coleoptera: Scolytideae) with its symbiotic fungus, *Ambrosiella hartigii; Ann. Entomol. Soc. Am.* **65** 719–721

French J R J and Roeper R A 1973 Patterns of nitrogen utilization between the ambrosia beetle *Xyleborus dispar* and its symbiotic fungus; *Insect Physiol.* **19** 593–605

Gadd C H and Loos C A 1947 The ambrosia fungus of *Xyleborus fornicatus* Eichh; *Trans. Br. Mycol. Soc.* **31** 13–18

Kalshoven L G E 1958 Studies on the biology of Indonesian Scolytoidea; *Entomol. Ber.* **18** 147–193

Kaneko T 1965 Biology of some scolytid ambrosia beetles attacking tea plants. I. Growth and development of two species of scolytid beetles reared on sterilized tea plants; *Jpn. J. Appl. Entomol. Zool.* **9** 211–216

Kok L T and Norris D M 1973 Comparative sterol compositions of adult female *Xyleborus ferrugineus* and its mutualistic fungal ectosymbionts; *Comp. Biochem. Physiol.* **B44** 499–505

Lindgren B S and Borden J H 1983 Survey and mass trapping of ambrosia beetles (Coleoptera: Scolytideae) in timber processing areas on Vancouver Island; *Can. J. For. Res.* **13** 481–493

Lodder J 1970 (ed.) *The yeasts, a taxonomic study*; (Amsterdam: North-Holland Publishing Co.) pp. 1385

Magema N, Gasper C and Severin M 1982 Efficacity of ethanol for trapping *Trypodendron linneatum* (Coleoptera: Scolytideae) and the influence of the *Picea terpenics; Ann. Soc. R. Zool. Belg.* **112** 49–60

Martin M M 1984 The role of ingested enzymes in the digestive processes of insects. In *Invertebrate-microbial interactions* (eds) J M Anderson, A D M Rayner and D W H Walton (London: British Mycological Society Symposium 6) 155–172

Muthappa B N and Venkatasubbaiah P 1981 Association of *Ambrosiella macrospora* with *Xylosandrus compactus*, the shot-hole borer of robusta coffee (*Coffea canephora*) in India; *J. Coffee Res.* **11** 54

Nakashima T 1972 Notes on the mycetangia of the ambrosia beetles, *Platypus severni* Blandford and *P. calamus* Blandford (Coleoptera: Platypodideae); *Appl. Entomol. Zool.* 7 217–225

Nakashima T 1975 Several types of the mycetangia found in platypodid ambrosia beetles (Coleoptera: Platypodideae); *Insecta Matsumurana NS* 7 1–69

Nakashima T 1979 Notes on the time when the new female adults of the ambrosia beetle *Crossotarsus niponicus* Blandford (Coleoptera: Platypodideae) harvest their symbiotic fungi into their mycetangia; *Insecta Matsumurana. NS* **17** 1–19

Norris D M and Baker F M 1969 Nutrition of *Xyleborus ferrugineus*. I. Ethanol in diets as a tunnelling (feeding) stimulant; *Ann Entomol. Soc. Am.* **62** 592–594

Nobuchi A 1972 The biology of Japanese Scolytideae and Platypodideae (Coleoptera); *Rev. Plant Prot. Res.* **5** 61–75

Schneider L A and Rudinsky J A 1969a Mycetangial glands and their seasonal changes in *Gnathotrichus retusus* and *G. sulcatus; Ann. Entomol. Soc. Am.* **62** 39–43

Schneider L A and Rudinsky J A 1969b Anatomical and histological changes in internal organs of adult *Trypodendron lineatum, Gnathotrichus retusus*, and *G. sulcatus* (Coleoptera: Scolytideae); *Ann. Entomol. Soc. Am.* **62** 995–1003

Singh P 1977 *Artificial diets for insects, mites and spiders*; (New York: Plenum Press) pp. 494

Weber B C and McPherson J E 1983 Life history of the ambrosia beetle *Xylosandrus germanus* (Coleoptera: Scolytideae); *Ann. Entomol. Soc. Am.* **76** 455–462

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 149–163.
© Printed in India.

Ecology of keratinophilic fungi

A P GARG*, SUDHA GANDOTRA, K G MUKERJI and G J F PUGH**

Department of Botany, University of Delhi, Delhi 110 007, India

*Department of Botany, I.P. College, Bulandshahr 203 001, India

**Department of Biological Sciences, Portsmouth Polytechnic, Portsmouth, Po1 2DY, UK

Abstract. The soil keratinophilic fungi are responsible for the breakdown of any keratin containing wastes such as hair, fur and feather. These fungi therefore play a significant role in the breakdown of soil debris. In this review we have evaluated the information available on ecological factors governing the distribution and growth of keratinolytic fungi.

Keywords. Keratin; Keratinophilic fungi; dermatophytes.

1. Introduction

With the introduction of hair-habit technique (Vanbreuseghem 1952) more precisely the To-Ka-Va hair baiting method (Benedek 1962) the long predicted natural saprophytic stage of dermatophytes in the soil was investigated throughout the world and reviewed by several workers (Ajello 1974; Hejtmanek *et al* 1973; Orr and Kuehn 1972; Otčenášek *et al* 1967; Palsson 1968; Piontelli and Caretta 1974; Volz 1975). Geographical distribution of pathogenic dermatophytes (Ainsworth and Austwick 1973; Ajello 1960, 1974; Canizares 1976; Palsson 1968; Philpot 1978; Vanbreuseghem 1970) indicates that soil acts as a reservoir for primary infection, at least for some pathogenic fungi, and also for those which are potentially pathogenic to small and big wild animals which are the source of secondary infection to man and various pets (Ajello 1953, 1954, 1956, 1959, 1974; Alteras and Evolceanu 1967; Alteras *et al* 1984; Georg 1959; Gip and Hersle 1966; Graham-Jones 1968; Simordova 1971; Stepanischcheva and Malkina 1971). Besides soil, birds' feathers and their nests (English and Morris 1969; Hubálek 1972, 1974, 1976a,b; Hubálek and Munro 1973; Otčenášek *et al* 1967; Pugh 1964, 1965, 1966a,b, 1972; Rees 1967b; Sur and Ghosh 1980), hairs of wild animals (Alteras *et al* 1966, 1984; Marples 1968; McKeever *et al* 1958; Rees 1967a,b; Saez and Chauvier 1977; Salebiau and Carlas 1980), water (Simordova and Hejtmanek 1970), plant debris (Balbanoff and Usunov 1967) and dung (Caretta and Frate 1976; Caretta *et al* 1976) are the different ecological nitches for the survival of potential dermatophytes.

Keratin, an important natural material occurring in nature mainly in the form of hair, feather, wool, horn, hoof, nails, and cornified epidermis, is difficult to be decomposed by dominant soil fungi (Mathison 1964; Pugh *et al* 1984). It is an important biodeteriorant because of its implications in wool industry and medical mycology. Any fungus which can produce enzymes capable of decomposing keratinized structures must be regarded as keratinophilic. When the pathogenic strains, surviving in soil, find a suitable host in favourable environmental and physiological conditions, they produce the symptoms of what is known as "ringworm diseases".

The literature in medical mycology is substantive and full of reports on the

geographical distribution and problems related to the taxonomy of these fungi, however, the ecological factors governing their distribution and survival in soil have attracted little attention from workers in the past. Keeping in view their great importance in biodeterioration of keratinous substrates and prevalence of dermatophytic infections in human and animal populations it has been thought desirable to present this review.

Various factors affecting the distribution and survival of keratinophilic fungi can be grouped under two categories, abiotic such as soil, temperature, light, seasonal variations, etc. and biotic such as wild animals and birds. The impact of each of these factors on the growth and survival of keratinophilic fungi is discussed in detail.

2. Climatic factors

2.1 *Temperature*

Keratinophilic fungi are generally considered to be mesophilic in nature though some species have been reported to be growing even at 37°C such as *Chrysosporium keratinophilum, C. tropicum, C. queenslandicum* etc., however, most of the geophilic dermatophytes grow best at 25–30°C. Pugh and Evans (1970) have reported optimum range of temperature as 25–27°C for most of the keratinophilic fungi and did not find any growth above 40°C.

The widely accepted view about their mesophilic nature has been disqualified by the findings of Battelli *et al* (1978) who reported the occurrence of *Microsporum gypseum, Trichophyton ajelloi, T. terrestre* in alpine mountain soils. Pugh and Allsopp (1982) have reported a frequent presence of *Chrysosporium pannorum* and *Mortierella* spp. with relatively rare occurrence of *Trichophyton terrestre* and *Chrysosporium* sp. (cf. *C. asperatum*) in Antarctica soils. Besides these an unidentified species of *Trichophyton* had also been isolated from keratinous substrates of Signy Islands (Antarctica). Recently, Pursola and Guarro (1984) have reported a new psychrophilic species *Keratinomyces ceratinicus* from forest soils in Spain, optimum temperature for the growth of this fungus in the laboratory being 15–17°C. Saez and Chauvier (1977) reported the highest temperature in which these fungi could survive as 33–41°C and the least thermotolerant strains were *Chrysosporium* sp. and *Trichophyton terrestre* while the most thermotolerant was *Chrysosporium keratinophilum*. Saez and Battesti (1970, 1972) reported the following temperature maxima for the survival of seven species of keratinophilic fungi (table 1).

Table 1. Temperature maxima for seven species of keratinophilic fungi (Saez and Battesti 1972).

Species	Temperature maxima (°C)
T. mentagrophytes	42
Epidermophyton floccosum	41
Microsporum canis	41
T. interdigitale	40
Trichophyton rubrum	40

Garg (unpublished work) investigated the optimum temperature for the growth of some of the keratinophilic fungi and their respective temperature maxima (table 2).

Thus, it can be said, that the keratinophilic fungi, in general, are mesophilic but some strains are thermotolerant and recent reports indicate that they can adapt themselves to adverse temperatures for their survival (Pugh and Allsopp 1982; Pursola and Guarro 1984).

2.2 *Light*

Far UV light is fungicidal as it brings about an inhibition of spore germination and eventually hyphal growth. Effect of quality of light on spore germination of dermatophytic species has been exclusively studied by Buchníček (1966, 1967, 1969, 1971, 1973, 1974, 1978, 1981).

Growth inhibition of dermatophytes by carbon filament lamp was first shown by Berde (1929). A fungicidal effect in *Candida albicans* was observed by Dickey (1962) which varied with the concentration of photosensitizer and the light intensity. Illumination of spore suspension of *T. mentagrophytes* without photosensitization had, however, no effect (Dickey 1962; Buchníček 1968). An inhibition of spore germination by visible light (400–700 nm) in little doses has been reported (Buchníček 1968). In the course of time it first becomes fungistatic and later fungicidal. The decline of the frequency of dermatomycoses in summer months could partly be due to this radiation effect. Long wavelength UV radiations inhibit germination and growth of dermatophytes, the intensity of inhibition being dependent on the length of daily exposure (Buchníček 1967). The susceptibility of spores to UV radiation varies from species to species, with *Keratinomyces ajelloi* complete lethal effect is achieved with a daily irradiation lasting 2 hr, with *M. cookei*, 1–2 hr, *T. mentagrophytes* var. *interdigitale* 1 hr (Buchníček 1967) while with *T. verrucosum* only 8 min exposure seems to be fatal (Buchníček 1969).

Visible light is known to inhibit growth of microcolonies up to 32–47%, red light 40–62% and blue light 80–94% (Buchníček 1971) while with green light 30–80% growth inhibition has been recorded (Buchníček 1973). Resistance to illumination varies with visible light also. The red or blue light when applied separately inhibit the growth more than the visible light. Also, the sum of inhibitions caused by both the colour lights applied separately is greater than the inhibition caused by their spectral mixture. Macroconidia of *M. gypseum* do not germinate and are damaged under the effect of UV and visible light (Buchníček 1981). The fact that growth of dermatophytes is

Table 2. Temperature optima and maxima for the growth of a few keratinophilic fungi (Garg, unpublished data).

Species	Temperature (°C)	Temperature maxima (°C)
Trichophyton ajelloi	25–28	35
T. terrestre	24–26	30
Ctenomyces serratus	22–26	30
Microsporum gypseum	28–30	38–40
Chrysosporium pannorum	21–24	28–30
C. keratinophilum	25–30	40

inhibited by near UV and visible light may have some epidemiological consequences in nature. Uncommon occurrence of dermatophytic infections in tropical countries particularly in summer than in temperate countries and in winter may also be due to the differences in solar radiations besides several other factors.

2.3 *Seasonal variations*

A change in the dermatophytic flora of the soil with season is quite anticipated since any climatic variations would lead to a corresponding change in temperature and radiations. Staib and Evangelinos (1968) isolated *Keratinomyces ajelloi*, *Microsporum gypseum* and *Trichophyton terrestre* in 23 % of soil samples during April while in August of the same year they found *M. cookei* in addition to the above species in 56 % of the soil samples collected from fifty different localities in Wuerzberg.

Seasonal climatic variations are sometimes well marked particularly in tropical and subtropical countries, therefore, they would naturally alter the course of succession, distribution and survival of the micro-organisms. Workers in the past while reporting on geophilic distribution of these fungi have not bothered about the seasonal variations, therefore, it is suggested that in future studies the geographical distribution should be investigated in different seasons to obtain a comprehensive picture of their distribution.

3. Soil factor

The process of keratin decomposition is a very complex one and the survival and persistence of keratinolytic fungi in soil is under the influence of various factors that interact mutually to control the dermatophytic population of the soil. These different parameters are discussed here in detail.

3.1 *Soil pH*

Effects of extreme pH values on microbial life is a very well explored subject. Regarding keratinophilic fungi Böhme and Ziegler (1965) made the first observation as to their occurrence with respect to soil pH which was later confirmed by Pugh (1966b). Ziegler (1966) studied the physiology of decomposition of keratin over a wide range of pH. His studies revealed the optimum pH for amylase—7·2, alkyl phosphatase—8·7, lipase—6·8, proteinase—5·4–6·9 and keratinase as 9. Ectoenzymes of dermatophytes are rendered inactive at pH below 4·5 and the enzymatic catabolism of keratinophilic fungi can only take place at pH 6·9.

Hubálek (1974) carried out an extensive study on the occurrence of keratinophilic fungi at different substrate pH values and hence categorized them into three groups: (i) acidophilic such as *Arthroderma uncinatum*, *A. curreyi* and *Chrysosporium tropicum* in acidic soils; (ii) neutrophilic mainly *Nannizia incurvata* in near neutral soils; and (iii) alkalophilic such as *A. quadrifidum*, *Ctenomyces serratus* and *Chrysosporium keratinophilum* in alkaline soils. Earlier Pugh (1966b) described *C. serratus* with the fungi of Hubálek's second category while *T. ajelloi* in the first. Their grouping in these categories has further been confirmed by Chmel and Vláčilíková (1977).

Figure 1 clearly indicates the pH optimum for the growth of a specific keratinophilic fungus and its possible occurrence on either side of the pH scale. The figure is based on

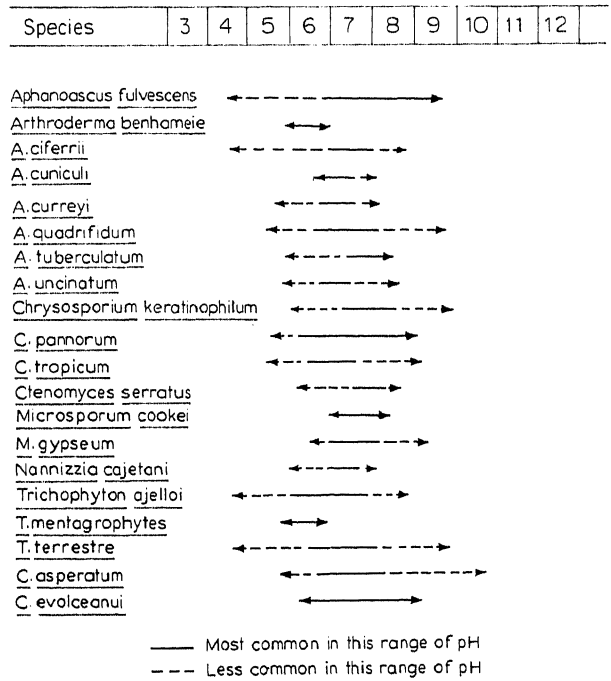


Figure 1. Distribution of geophilic dermatophytes in relation to soil pH.

investigations up to 1983. Since one encounters great variations in pH of keratinous substrates colonized, it is of utmost significance to study the geographic distribution of these fungi in relation to substrate pH. As indicated by Ziegler (1966) and further confirmed by numerous workers, the most luxuriant growth and highest frequency of keratinophilic fungi is found in soils with pH 6–9.

3.2 *Carbonate*

Though the keratinophilic fungi can degrade highly complex carbon compounds there is little information about carbonate affecting their distribution in soils. Chmel *et al* (1972) have reported a preferential occurrence of *M. gypseum* in carbonate meadow soils, a loose association of *K. ajelloi* with such soils and a preference of *T. terrestre* for alluvial soils. Chmel and Vláčilikóva (1977) reported an abundance of keratinophilic fungi in carbonate meadow and chernorzemic soils with higher humus content than in grey podzolic soils. It seems likely that keratinophilic fungi can degrade highly complex carbon compounds in various culture media, therefore a simple carbon source which can easily be utilized may not affect their qualitative distribution but the studies on their quantitative distribution may reveal quite interesting facts.

3.3 *Nitrogen*

Keratin is a rich source of nitrogen and can only be decomposed by keratinolytic fungi (Kuester and Safwat 1978). A higher rate of keratinolysis has been reported by them at pH 6–9 thus giving support to an earlier finding of Ziegler (1966).

Importance of nitrogen in the growth of keratinophilic fungus *Microsporum gypseum* is very well illustrated by the experiments employing optically different forms of cystine, which is found in relatively large proportions in the scleroprotein (Kunert 1982). A rapid utilization of carbon and nitrogen of L-cystine was observed while excess of sulphur was secreted into the medium where it got oxidized to sulphate. On the other hand, oxidation of sulphur in the extracellular medium using DL-cystine was relatively low indicating a slower rate of uptake of this form. However, the mode of utilization of L- and DL-cystine was same.

The nitrogenous content of keratin varies from one form to another and this in turn would affect colonization of the latter by keratinophilic fungi.

3.4 *Sulphur*

Kunert (1981a,b) studied the inorganic and organic sulphur sources for the growth of the fungus *M. gypseum.* Sodium-sulphate, sulphite, disulphite, peroxodisulphate and dithionate were the best sources of inorganic sulphur. Of the inorganic sources sulphide caused an initial inhibition of the fungal growth supporting an earlier report of Furuya (1976) that mineral waters containing H_2S are inhibitory for the growth of dermatophytes. Amino acids such as cystine, cysteine, glutathione, S-sulphocysteine, lanthioneine, taurine, and serine-sulphate were the best organic sulphur sources for the fungus. Growth was slow with organic sulphur compounds other than amino acids. Sodium dodecyl sulphate completely inhibited the germination and growth of this fungus (Kunert 1981b). A higher rate of utilization of cystine by keratinophilic fungi than that of methionine (Kunert 1981b) can be correlated with that of the composition of their natural substrate, keratin. Cystine with disulphide bridges constitutes about 12–16% of total scleroprotein in highly keratinized structures while methionine is present in lesser amounts. Sulphur availability, thus, has a significant bearing on the distribution of keratinolytic fungi.

3.5 *Moisture content*

Water constitutes a large proportion of the cell cytoplasm and is vital to life since most of the enzymatic reactions occur in aqueous solution. The moisture availability to fungi living in soil varies with habitats. The processes of germination, growth and reproduction are directly related to the substrate moisture content. Although, vast literature is available relating the incidence of fungi with moisture availability very little information is known to show its effect on the decomposition of keratinous substrate. Pugh and Evans (1970) reported a higher percentage of spore germination in *Arthroderma uncinatum* and *Ctenomyces serratus* at 90–100% relative humidity. They also reported an abundance of keratinolytic fungi in birds' nests (soil) with moisture content generally below 10%. Hubálek and Munro (1973) showed a greater incidence of keratinophilic fungi in birds' nests with 15–20% water content. Hubálek (1976) reported the occurrence of *A. curreyi* in nests with 11·81% and 19·85% water content while *C. keratinophilum* was isolated rather consistently from the nests with higher moisture content thereby indicating its hygrophilic nature. Hubálek and Balat (1976) on further examination of the nests of tree sparrow (*Passer montanus* L.) found *C. keratinophilum*, and *A. fulvescens* as hygrophilic in nature. A direct relationship between the number of isolates of *K. ajelloi* of soil community and that of the moisture content of soil was found by Chmel *et al* (1972). This fungus occurred in soils with 16·2–17·8%

water content. *T. terrestre* was isolated in greater abundance from the soil samples with an average water content of 7·1 % decreasing with increase in average water content. These findings lead us to conclude that *K. ajelloi* is hygrotolerant while *T. terrestre* is xerophilic in nature.

From the foregoing account it becomes clear that the keratinophilic fungi are in general mesophilic with a few exceptions of hygrophilic and xerophilic species. Since the seasonal variations influence to a large extent the percent water content of the soil, the absence or presence of a specific keratinolytic fungus may be due to the available water content at a particular time of the year. However, this is only one of the factors which influence the colonization and course of succession on keratin rich substrates.

3.6 *Humus*

The first report as to the occurrence of *M. gypseum* preferentially in soils with high humus content (5·1 %) and that of *T. terrestre* in soils with low humus (1·5 %) was presented by Chmel *et al* (1972). However, distribution of *K. ajelloi* and *C. keratinophilum* is irrespective of soil humus (Chmel *et al* 1972; Schönborn 1968). Ajello and Padhye (1974) reported the occurrence of *A. quadrifidum*, *C. indicum*, *C. keratinophilum*, *C. tropicum* and *Ctenomyces serratus* in soils made up of disintegrated lava with low organic matter in the Galapagos Islands. Chmel and Vlačilikova (1975) reported a higher number of keratinophilic fungi in soils with high humus content. *M. gypseum* although present in all the four layers of soil profile was maximum in the zone with high humus content. *T. georgii* was present exclusively in soils with high humus content while *T. vanbreuseghemii* and its perfect state (*A. gertleri*) was found mainly in soils with low humus content. Thus we can form two groups of keratinophilic fungi based on their preference for humus, first exclusively associated with humus and second showing no distinct relationship.

Balbanoff and Usunov (1967) reported *T. terrestre*, *A. quadrifidum*, *K. ajelloi*, *M. cookei* and *M. gypseum* from plant waste organic matter constantly contaminated by animals. However, this was the only report concerning isolation of keratinophilic fungi from natural organic waste matter of plant origin. It is safe to believe that the very presence of these fungi was due to contamination of plant debris by infected keratinous substrates. It has been observed that infected hairs with pathogenic dermatophytes generally drop down on the soils and the collection of the latter lead to erroneous results.

3.7 *Heavy metals*

Williams and Pugh (1974) have reported the resistance of *C. pannorum* to high concentrations of an organic mercurial preparation. Artis *et al* (1983) reported the dependency of *T. mentagrophytes* ATCC-18748 on iron. Depletion of iron from the medium resulted in an inhibition of the growth of spores beyond germ tube formation. Pugh *et al* (1984) have reported that *C. keratinophilum* is resistant to very high concentration of nitrates (2000 mg/k) of Cu, Zn and Pb. They have found that increasing concentration of heavy metals decrease the tyrosine release indicating their possible effect on proteinase activity. Pugh *et al* (to be published) have found that geophilic keratinophilic fungi are generally tolerant to the levels of heavy metals found in heavily contaminated soils and thus confirming the reports of dermal infections in mine workers.

From the foregoing account it is evident that the keratinophilic fungi and allied dermatophytes have adapted themselves to the concentration of heavy metals found naturally in soils. This might be due to a higher accumulation of these metals in their hyphae.

3.8 *Fatty acids and oils*

Antimycotic properties of fatty acids were known to the scientific world as early as 1899 with the discovery of Clarke but the antidermatophytic properties contained in them were discussed only in 1940 by Rigler and Greathouse. This was further confirmed by Das and Banerji (1982, 1983); Hellgreen and Vincent (1976); Linderberg and Linderberg (1974); Nathanson (1960); Wyss *et al* (1945). Garg *et al* (unpublished data) pointed out some inherent defects employed by these workers in their studies and developed a new technique emphasizing that the use of an ultrasonicator or "Polytron" to incorporate oils and fats into the culture media. With this improved technique, they have demonstrated a marked fungistatic activity of some naturally occurring fatty acids and Indian hair oils at a very low concentration.

Hajini *et al* (1970) tested unsaturated fatty acids, hair oils, and various natural fatty acid products for their anti-dermatophytic properties. They reported an inhibition of growth of *T. rubrum* and related dermatophytes at 1 % concentration of mustard oil, oleic, linoleic, linolenic, and arachidionic acids while coconut oil, caster oil, till oil, Bryl cream, vaseline hair tonic, palmitic acid and stearic acid did not inhibit the growth even at 10% strength.

Since the occurrence of acetic, propionic, caproil, caprylic, lactic, ascorbic, pelargonic, tridecanoic, oleic, linolenic, myristic, palmitic and stearic acids in sebum, sweat, animal hair, wool feathers and other keratinous substrates is very well established, it is of utmost importance that while studying the growth of these fungi, keratinous bait must be defatted so as to ensure a proper growth of the fungus. The type and amount of fatty acids present in the soils affect the distribution and survival of keratinophilic fungi and allied dermatophytes.

3.9 *Salts*

Keratinophilic fungi can survive in a variety of habitats in coastal soils (Pugh and Mathison 1962). In mud both uncolonized and colonized by higher plants, *A. curreyi* has been reported to occur though the rate of accessibility of keratin must be small, which is subject to removal by tides. Species isolated from sand dunes inhabited by hares, rabbits and birds were *A. curreyi* and *Ctenomyces serratus* (Pugh and Mathison 1962). Padhye *et al* (1967) isolated *Chrysosporium tropicum* and *Microsporum gypseum* from long submerged marine soils in Bombay. *C. indicum* and *Ctenomyces serratus* have also been isolated but rather infrequently.

The salinity tolerated by these fungi remains to be determined, for no such fungi have been isolated from coastal Mediterranean soils which have salinity of the order of 5·8 to 15·6 %. An inhibitory effect of sodium chloride on the growth of dermatophytes has been reported. (Orrú *et al* 1968). Fungi whose growth was inhibited by NaCl were *Microsporum, Epidermophyton* and *Trichophyton.*

The foregoing account clearly indicates that keratinophilic fungi do colonize marine and terrestrial salt habitats but their tolerance to salts is very limited. The sensitivity of various species to salt concentrations varies to a great deal.

3.10 *Agrochemicals*

Increased world population has resulted in multitude of problems one of which is food storage. This has invited the use of chemicals in modern agricultural practices and one of the inherent defects is the biomagnification of the products of their decomposition which are toxic to life. The magnitude of toxicity becomes manifold and disturbs the ecological balance of nature. This is also one of the causal factors involved with the extinction of species.

There are numerous reports on the growth, and inhibition of various soil fungi by common herbicides and fungicides (Agnihotri 1971; Kuthubutheen and Pugh 1979; Pugh and Williams 1971; Wilkinson and Lucas 1969). Pugh and Williams (1971) found that the application of fungicide, Verdasan results in a rapid reduction of soil fungal population. Inhibition of growth of *T. ajelloi*, the most common fungus of temperate soils associated with acidic conditions by Vardasan has been reported by Pugh and Agrawal (1983). It is effective even at concentration of 5–10 ppm. This fungus has been found to be somewhat tolerant to lower doses of captan (N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide) which could be attributed to the fungistatic nature of this compound.

It is of interest that *T. ajelloi* grows better and degrades keratin faster when there is less competition in soil and since with increasing use of agrochemicals the soil fungal population decreases. The soil may become a reservoir of these potential pathogens that are resistant to pesticides and from which infection to man and animals could result.

3.11 *Substrate*

Keratinophilic fungi inhabit a variety of ecological sites frequented by animals and man such as, playgrounds, recreation centres, poultry farms, cattle pans, stables, zoological parks, swimming pools, and other places frequented by animals and man. In whatever habitat they occur, they are a group of botanically related fungi with affinity for epidermal scales and hairs. Although Balbanoff and Usunov (1967) have reported *T. terrestre*, *M. gypseum* from plant debris but their non-keratinous nature is still doubtful. Thus, keratin is the major limiting factor contributing to their distribution. Various forms of keratin have been used in culture studies of keratinophilic fungi, the more common ones being animal hair, wool, nails, feathers, horns, hoof, skin scrappings and cornified epidermal tissue. Hair as culture medium for dermatophytes was introduced by Sabouraud, however, the much used To-Ka-Va hair baiting technique came into being in 1952 only after a series of progress was made in this direction by Toma (1929), Karling (1946) and Vanbreuseghem (1952). Since that time various types of keratinous baits have been used to isolate keratinophilic fungi from soils.

The quality of bait has also been worked upon. Various forms of keratin have been used in cultures to estimate their potential to serve as substrate for keratinophilic fungi. Knudsen (1980) has reported the survival of *E. floccosum*, *M. canis*, *T. rubrum*, *T. mentagrophytes* on skin scrappings. Mercantini *et al* (1983) while surveying soils of public gardens and parks in Rome found that the keratinous substrate in soil definitely influences the biological cycle of these fungi. Battelli *et al* (1978) reported a higher occurrence of *M. gypseum* in the burrows of alpine marmat than in the adjoining areas.

Thus, it is evident that animalization, that enrichment of soil with keratinous organic

matter may create suitable conditions for growth of keratinophilic fungi.

3.12 *Competition*

Since keratinophilic fungi have been reported to grow well in semi-synthetic media, it is very likely that they grow, and sporulate in soil with known keratinous bait but it is definite that they are poor competitors for such simple substances while many fungi in soil show optimum growth under the same condition. The competition is minimized owing to the exceptional characteristic of these fungi to secrete keratinolytic enzymes while other fungi are unable to do so.

It is rather difficult to visualize how these fungi with complex systems of enzymes in them, are not able to grow in the presence of simple cellulosic and proteinaceous compounds, which may be attributed to their poor competitive ability. Relative rare occurrence of zoophilic and anthropophilic dermatophytes in the soils can also be attributed to their poor competitive ability. The cosmopolitan nature of *T. ajelloi*, *T. georgii*, *T. rubrum*, *T. schöenleinii*, *T. terrestre*, *T. tonsurans*, *T. verrucosum*, *T. violaceum* and *T. gallineae* may be because of their relatively good competitive ability.

4. Biotic factors

The biotic component influences the occurrence of keratinophilic fungi to a great extent and is the chief contributory factor in transmission and persistence of ringworm diseases caused by dermatophytes. The two complements of the biotic component which influence the occurrence of these fungi are birds and animals.

4.1 *Birds*

The association, survival and dispersal of keratinophilic fungi and allied dermatophyte with birds' feathers has been recognised by various authors (Ajello 1953; Bühlmann and Reith 1962; Gierløff and Katić 1961; Hubálek 1972, 1974, 1976a, b; Hübschmann and Frágner 1962; Kuehn 1960; Pugh 1966a; Pugh and Evans 1970; Rees 1967a, b).

Pugh (1965) showed the presence of keratinophilic fungi on 36·6% of the experimental birds while Rees (1967a) recorded them on 40% birds in Australia. A close relationship exists between the keratinophilic fungi and the birds of a specific order, *e.g.* *A. curreyi* and *Turdus* (Pugh 1964); *C. serratus* and members of Galliforme, especially partridges (Pugh 1966a) and chickens (Rees 1967b), *Chrysosporium* spp. have been very frequently reported from most of the bird types (Pugh 1965, 1966a; Rees 1967a, b).

High frequency of keratinophilic fungi have been found in birds' nests. More commonly found species are *C. keratinophilum*, *Keratinomyces ajelloi* and *T. terrestre* (Otčenášek *et al* 1967; Pugh 1966b). Hubálek (1975) has specified hen habitat for the cleistrocarpic stage of *C. serratus*.

4.2 *Animals*

Animals, be small or large, serve as an important source of secondary infection of dermatophytes in man. The role of wild animals in carrying over these diseases has been recognized by Alteras *et al* (1966), English (1967), Gugnani *et al* (1967, 1971), Hoffman *et al* (1970) and McKeever *et al* (1958).

Alteras *et al* (1984) reported the occurrence of *Tinea capitis* in 2-month-old and

11-month-old infants who acquired this infection from stray kittens further supporting the view that wild animals cause secondary infection in man. Occurrence of scalp lesions in an 8-year-old boy and a 32-month-old yolkshire saw and consequently isolation of *M. nanum* from soils show that soil acts as a reservoir for dermatophytes from which primary and secondary infections may result (Ajello *et al* 1965).

Mariat *et al* (1976) reported 15·78% of mammals contaminated by dermatophytes. *M. persicolor* was found mainly on male bank voles and *T. mentagrophytes* on male wood mice. *T. mentagrophytes* has been known from soils from where primary infection might have resulted on wood mice that becomes the source of secondary infection for higher mammals. Species like *T. terrestre*, *K. ajelloi*, *C. keratinophilum*, *Chrysosporium* sp. and *Scopulariopsis bevicolis* have been reported to prevail on subhuman primates and monkeys (Saez and Chauvier 1977). Otčenášek *et al* (1967) reported *M. persicolor*, *T. georgii*, *T. mentagrophytes* var. *erinaci*, *T. mentagrophytes* var. *mentagrophytes* and *T. terrestre* from 32·9% of their experimental animals mainly insectivores and rodents showing otherwise healthy symptoms. In a survey of wild animals, carried out by Mantovani *et al* (1982) it was found that *T. mentagrophytes* var. *mentagrophytes* was mainly isolated from rodents, *T. mentagrophytes* var. *erinaci* from rodents living in anthropesized areas while *M. gypseum* from all types of animal habitats with the exception of Chiropteran habitats. *Chrysosporium* spp. have been reported in sheep, horse, cattle, pigs and goat-habitats and *Histoplasma capsulatum* in Chiropteran habitats (Ajello *et al* 1965). It may be concluded from these observations that wild animals not only act as carriers of dermatophytic fungi but also provide a suitable habitat for their survival as saprophytes.

5. Conclusion

It is evident from the above account that keratinophilic fungi and allied dermatophyte survive saprophytically on soil and wild animals including birds and humans which provide an inoculum for primary and secondary infections to various animals and humans. Keratin a natural complex proteinaceous substrate is decomposed by the keratinolytic activity of these fungi. Various ecological factors affect and control their survival, distribution, pathogenesis and keratinolytic activity.

References

Agnihotri V P 1971 Persistence of captan and its effects on microflora, respiration and nitrification of a forest nursery soil; *Can. Microbiol.* **17** 377–383

Ainsworth G C and Austwick P K C 1973 Fungal diseases of animals; *Rev. Ser.* 6 *CAB* England

Ajello L 1953 The dermatophyte *M. gypseum* as a saprophyte and parasite; *J. Invest. Dermatol.* **21** 157–171

Ajello L 1954 Occurrence of *Histoplasma capsulatum* and other human pathogenic molds in Panamanian soils; *Am. J. Trop. Med. Hyg.* 3 897–904

Ajello L 1956 Soil as a natural reservoir for human pathogenic fungi; *Science* **123** 876–879

Ajello L 1959 A new *Microsporum* and its occurrence in soil and on animals; *Mycologia* **51** 69–76

Ajello L 1960 Geographic distribution and prevalence of the dermatophytes; *Ann. N. Y. Acad. Sci.* **89** 30–38

Ajello L 1974 Natural history of the dermatophytes and related fungi; *Mycopathol. Mycol. Appl.* **53** 93–110

Ajello L and Padhye A 1974 Keratinophilic fungi of the Galapagos Islands; *Mykosen* **17** 239–243

Ajello L, Varsavsky E, Satgin G, Mazzoni A and Mantovani A 1965 Survey of soils for human pathogenic fungi from the Emilia-Romagna region of Italy. I. Isolation of keratinophilic fungi; *Mycopathol. Mycol. Appl.* **26** 65–71

Alteras I and Evolceanu R 1967 Human infections by *Microsporum gypseum* in Romania; *Mycopathol. Mycol. Appl.* **33** 140–144

Alteras I, Nesterov V and Clolofan I 1966 The occurrence of dermatophytes in wild animals from Romania; *Sabouraudia* **4** 215–218

Alteras I, Feuerman E J, Grunwald M and Shvili D 1984 Tinea capitis due to *Microsporum canis* in infants; *Mycopathologia* **86** 89–92

Artis W M, Wade T R and Jones H E 1983 Restoration of *Trichophyton mentagrophytes* growth in medium depleted of metals by chelation. Importance of iron; *Sabouraudia* **21** 41–48

Balbanoff V A and Usunov P 1967 Organic waste matter of plant origin—natural source of primitive dermatophytes. I. Communication; *Mycopathol. Mycol. Appl.* **33** 43–48

Battelli G, Bianchedi M, Frigo W, Amorati P, Mantovani A C and Pagliani A 1978 Survey of keratinophilic fungi in alpine marmat (*Marmata marmata*) burrow soil and in adjoining soils; *Sabouraudia* **16** 83–86

Benedek T 1962 Fragmenta mycologica. I. Some historical remarks on the development of "hair-baiting" of Toma-Karling-Vanbreuseghem (The To-Ka-Va hair baiting method); *Mycopathol. Mycol. Appl.* **16** 104–106

Berde K 1929 Verhalten von Fadenpilzkolonien unter der Wirkung des Lichter; *Arch. Dermatol. Syph.* **158** 35–50

Böhme H and Ziegler H 1965 Verbreitung und Keratinophilie von *Anixiopsis stercoraria* (Hansen) Hansen; *Arch. Klin Exp. Dermatol.* **223** 422–428

Buchníček J 1966 Über den einfluss der langwelligen UV-strahlung auf das wachstums von dermatophyten. III; *Symp. Int. Dermatol. Bratislava Abst.* 10–16

Buchníček J 1967 Über den einfluss des lichtes auf das keimen und wachstum von dermatophyten. I. Wiederholte exposition der UV-strahlung; *Acta Univ. Olomuc. Fac. Med.* **45** 141–165

Buchníček J 1968 Inhibition of germination in the spores of *Trichophyton mentagrophyta* caused by visible light; *Folia Fac. Ser. Nat. Brun.* **IX/5** 23–28

Buchníček J 1969 Inhibition des keimens und wachstums von *Trichophyton verrucosum* durch wiederholte beleuchtung; *Mykosen* **12** 321–328

Buchníček J 1971 Inhibierende einwirkung des blauen und roten lichtes auf *Trichophyton*; *Mykosen* **14** 393–397

Buchníček J 1973 Uber die wachstumshemmung der dermatophyten durch grunes licht; *Mykosen* **16** 327–331

Buchníček J 1974 Biologische inhibition durch licht; *Acta Univ. Olomuc. Fac. Med.* **71** 161–237

Buchníček J 1978 Morphological alterations in dermophytes inhibited by light; *Acta Univ. Olomuc. Fac. Med.* **79** 219–230

Buchníček J 1981 Die ultrastruktur der apikalzellen bei *Microsporum gypseum* nach wachstumshemmung durch ultraviolettes licht; *Mykosen* **24** 472–481

Bühlmann X and Rieth H 1962 Über die Erkennung und Bedeutung von Dermatomykosen bei Haustieren, Schweizer; *Arch. Tierheilk.* **104** 537–545

Canizares O 1976 Dermatology in India; *Arch. Dermatol.* **112** 93–97

Caretta G and Frate D 1976 Funghi cheratinofilie dell' isola di Montecristo: isolamenti da sualo, escrementi e pelo di animali e piume di uecelli; *Atti Ist. Bot. Univ. Lab. Crittogam Parlas* **6** 203–208

Caretta G, Frate G Del, Piontelli E and Todaro F 1976 Keratinophilic mycoflora of hair and dung of cows, in fodder and in the soil on farms: their distribution. *Riv. Parasitol.* **37** 333–361

Chmel L and Vlačiliková A 1975 The ecology of keratinophilic fungi at different depths of soil; *Sabouraudia* **13** 185–191

Chmel L and Vlačiliková A 1977 Keratinophilic fungi in some types of soil and factors influencing their occurrence; *Biologia* **32** 53–59

Chmel L, Hasilikova A, Hrasko J and Vlačiliková A 1972 The influence of some ecological factors on keratinophilic fungi in the soil; *Sabouraudia* **10** 26–34

Clark J F 1899 On the toxic effects of deleterious agents on the germination and development of certain filamentous fungi; *Bot. Gaz.* **28** 289–327

Das S K and Banerjee A B 1982 Effect of undecanoic acid on phospholipid metabolism in *Trichophyton rubrum*; *Sabouraudia* **20** 267–272

Das S K and Banerjee A B 1983 Effect of undecanoic acid on lipid composition of *Trichophyton rubrum*; *Mycopathologia* **83** 35–39

Dickey R F 1962 Investigative studies in fungicidal powers of photodynamic action; *J. Invest. Dermatol.* **39** 7–19

English M P 1967 Ringworm in wild mammals; *Jul. Zool.* **153** 556–561

English M P and Morris P 1969 *Trichophyton mentagrophytes* var. *erinacei* in hedgehog nests; *Sabouraudia* **7** 118–121

Furuya H 1976 Effect of hot spring waters on the growth of *Trichophyton mentagrophytes* and *Trichophyton rubrum*; *J. Dermatol.* 3 73–83

Georg L K 1959 Animal ringworm in public health; *U.S.D. Hlth. Edu. Wel.* pp. 57

Gierløff B, Chr H and Katić I 1961 Om anvendelse af griseofulvin specielt i veterinaer praksis; *Nord. Vet. Med.* **13** 571–592

Gip L and Hersle K 1966 Ringworm lesions caused by *Microsporum gypseum*. Two cases of soil infection. *Acta Dermatol. Venereol Stockh.* **46** 82–85

Graham-Jones O 1968 *Some diseases of animals communicable to man in Britain* (Oxford and New York: Pergamon Press)

Gugnani H C, Mulay D N and Murty D K 1967 Fungus flora of dermatophytosis and *Trichophyton simii* infection in North India; *Indian J. Dermatol. Vener.* **33** 73–82

Gugnani H C, Randhawa M S and Srivastava J B 1971 Isolation of dermatophytes and other keratinophilic fungi from apparently healthy skin coats of domestic animals; *Indian J. Med. Res.* **59** 1699–1702

Hajini G H, Khandari K C, Mohapatra L N and Bhutani L K 1970 Effect of hair oil and fatty acids on growth of dermatophytes and their *in vitro* penetration of human scalp hair; *Sabouraudia* **8** 174–176

Hejtmanek M, Hejtmankova N and Kunert J 1973 On the occurrence of geophilic dermatophytes in Asia; *Ceska Mykol* **27** 159–161

Hellgreen L and Vincent J 1976 Contact sensitizing properties of some fatty acids in dermatophytes; *Sabouraudia* **14** 243–249

Hoffman R, Kolipp D and Koch H A 1970 Significance of mice and other small mammals for distribution of dermatophytes and other keratinophilic fungus. Data on the epidemiology of dermatomycoses; *Mykosen* **13** 583–587

Hubálek Z 1972 Keratinophilic fungi on wild birds; *Mykosen* **15** 207–211

Hubálek Z 1974 Fungi associated with free living birds in Czechoslovakia and Yugoslavia; *Acta Sci. Natl Brun.* **8** 1–62

Hubálek Z 1976a Occurrence of keratinophilic fungi in nests of tree sparrow (*Passer montanus*) in relation to the substrate moisture; *Ceska Mykol* **30** 106–109

Hubálek Z 1976b Interspecific affinity among keratinolytic fungi associated with birds; *Folia Parasitol.* **23** 267–272

Hubálek Z and Balat F 1976 Seasonal distribution of keratinolytic fungi in the nests of tree sparrow (*Passer montanus* L.) Zentralbl Bakteriol Parasitenkol Infektionskr Myg Zweite-Naturwiss; *Abt Allg Landwirtsch Tech. Mikrobiol.* **131** 179–197

Hubálek Z and Rush-Munro F M 1973 A dermatophyte from birds: *Microsporum ripariae* sp. nov. *Sabouraudia* **11** 287–292

Hübschmann K and Fragner P 1962 In Dvorak J and Otcenasek M 1964 Geophilic, zoophilic and anthropophilic dermatophytes—A review; *Mycopathol. Mycol. Appl.* **23** 294–296

Karling J S 1946 Keratinophilic chytrids I; *Am. J. Bot.* **33** 751–757

Knudsen E A 1980 The survival of dermatophytes from tape strippings of skin; *Sabouraudia* **18** 145–148

Kuehn H M 1960 Observations on Gymnoascaceae. VIII. A new species of *Arthroderma*; *Mycopathol. Mycol. Appl.* **13** 189–197

Kunert J 1981a Inorganic sulphur sources for the growth of the dermatophytes *Microsporum gypseum*; *Folia Microbiol.* **26** 196–200

Kunert J 1981b Organic sulphur sources for the growth of the dermatophyte *Microsporum gypseum*; *Folia Microbiol.* **26** 201–206

Kunert J 1982 Utilization of L- and D-cystine by the fungus *Microsporum gypseum*; *Folia Microbiol.* **27** 390–394

Kuester E and Safwat 1978 Untersudrungen zur Kompostiesung von Federatefallen, studies on the compositing of features. Zbl. Batch II. *Abt., Bot.* **133** 385–393

Kuthubutheen A J and Pugh G J F 1979 The effects of Fungicides on soil fungal populations. *Soil Biol. Biochem.* **11** 297–303

Linderberg G and Linderberg M 1974 Effect of short chain fatty acids on the growth of some mycorrhizal and saprophytic hymenomycetes; *Asch. Microbiol.* **101** 109–114

Mantovani A, Morganti D, Battelli G, Mantovani A P, Poglayeni G, Tampieri M P and Vecchi G 1982 The role of wild animals in the ecology of dermatophytes and related fungi; *Folia Parasitol.* **29** 79–84

Mariat F, Jacqueline C and Marie-Ange R 1976 Study of contamination by dermatophytic fungi in a population of small wild mammals in Alsace; *Mycopathologia* **58** 71–78

Marples M J 1967 Nondomestic animals in New Zealand and Rasotonga as a reservoir of the agents of ringworm; *N.Z. Med. J.* **66** 1299–1302

Marples M J 1968 Some observations on the distribution of dermatophytes in Pacific Islands; *Bull. Pharma. Res. Inst. Tokatsuki* **72** 7–11

Mathison G E 1964 The microbiological decomposition of keratin; *Ann. Soc. Belgi Med. Trop.* **44** 767–792

McKeever S, Kaplan W and Ajello L 1958 Ringworm fungi of large wild animals in south western Georgia and north western Florida; *Am. J. Vet. Res.* **19** 973–975

Mercantini R, Marsella R, Capirilli F, Dovigiallo G 1980 Isolation of dermatophytes and correlated species from the soil of the public gardens and parks in Rome; *Sabouraudia* **18** 123–128

Nathanson R B 1960 The fungistatic action of oleic, linolenic acids on *Trichophyton rubrum* in vitro; *J. Invest. Dermatol.* **35** 261–263

Orr G F and Kuehn H H 1972 Notes on Gymnoascaceae. II. Some Gymnoascaceae and Keratinophilic fungi from Utah; *Mycologia* **64** 55–72

Orrú A, Pinetti P and Aste N 1968 Variazioni della frequenza di isolamento di dermatofili cheratinofili terricdi dalle sabbi di alcuni litorali della Sardegna centromeridionale nei diversi periodi dell anno; *Ras. Med. Sarda* **71** 665–676

Otčenášek M, Hudec K, Hubálek Z and Dvorak J 1967 Keratinophilic fungi from the nests in birds in Czechoslovakia; *Sabouraudia* **5** 350–354

Padhye A A, Pawen V H, Sukapure R S and Thirumalchan M J 1967 Keratinophilic fungi from marine soils of Bombay. India I. *Hind. Antibiotics Bull.* **10** 130–141

Palsson G 1968 Geophilic dermatophytes in the soil in Sweden. Studies on their occurrence and pathogenic properties; *Acta Vet Sci. and Suppl.* **25** 1–89

Piontelli E and Caretta G 1974 Considerazioni ecologische su Alconi gromiceti isolati su substrati cheratinici in localita montagnoe delle ande del cile; *Rev Pathol. Veg.* **10** 261–314

Philpot C M 1978 Geographical distribution of dermatophyte: A review; *J. Hyg.* **80** 301–314

Pugh G J F 1964 Dispersal of *Arthroderma curreyi* by birds, and its role in the soil; *Sabouraudia* **3** 275–278

Pugh G J F 1965 Cellulolytic and keratinophilic fungi recorded on birds; *Sabouraudia* **4** 85–91

Pugh G J F 1966a Fungi on birds in India; *J. Indian Bot. Soc.* **45** 296–303

Pugh G J F 1966b Associations between birds, nests, their pH and keratinophilic fungi; *Sabouraudia* **5** 49–53

Pugh G J F 1972 The contamination of birds of feathers by fungi; *Ibis* **114** 172–177

Pugh G J F and Agrawal S C 1983 Sensitivity of *Trichophyton ajelloi* to some common agrochemicals; *Mycopathologia* **81** 117–121

Pugh G J F and Allsopp D 1982 Microfungi on Signy Island, South Orkney Islands; *Br. Antarct. Surv. Bull.* **57** 55–67

Pugh G J F and Evans M D 1970 Keratinophilic fungi associated with birds. I. Fungi isolated from feathers, nests and soils; *Trans. Br. Mycol. Soc.* **54** 233–240

Pugh G J F and Mathison G E 1962 Studies on fungi in coastal soils. III. An ecological survey of keratinophilic fungi; *Trans. Br. Mycol. Soc.* **45** 567–572

Pugh G J F and Williams J I 1971 Effects of an organomercury fungicide on saprophytic fungi and on litter decomposition; *Trans. Br. Mycol. Soc.* **57** 164–166

Pugh G J F, Garg A P and Smith S N 1984 Inhibition of the keratinophilic fungus *Chrysosporium keratinophilum*. Int. Biodeterioration Conf. Washington D.C., U.S.A. (in press)

Pursola L and Guarro J 1984 *Keratinomyces ceretanicus* sp. nov., a psychrophilic dermatophyte from soil; *Mycopathologia* **85** 185–190

Rees R G 1967a Keratinophilic fungi from Queensland—I. Isolation from animal hair scales; *Sabouraudia* **5** 165–172

Rees R G 1967b Keratinophilic fungi from Queensland-II. Isolation from feathers of wild birds; *Sabouraudia* **6** 14–18

Rigler N E and Greathouse G A 1940 The chemistry of resistance of plants to *Phymatotrichum* root rot. VI. Fungicidal properties of fatty acids; *Am. J. Bot.* **27** 701–704

Saez H and Battesti M R 1970 Temperature maximale de development de quelques dermatophytes. Note preliminaire; *Parasitologia* **3** 179–183

Saez H and Battesti M R 1972 Growth of dermatophytes *in vitro* in the specific diagnosis of *Trichophyton unquinum*; *Ann. Dermatol. Syph.* **5** 501–509

Saez H and Chauvier G 1977 The prevalence of keratinophilic fungi on the hair of primates without clinical skin disorders; *Sci. Tech. Anim. Lab.* **2** 83–87

Salebiau A and Carlas da Silva L 1980 Isolation of dermatophytes from hair of wild animals; *An. Bras. Dermatol.* **55** 125–130

Schönborn C 1983 Zur vorkommen von *Chrysosporium keratinophilum* (Frey) Carmichael und einer thermophilen variante un Erdboden und bei Tieren; *Mykosen* **11** 847–864

Simordova M 1971 Contribution to the problem of the working environment in horticulture with regards to the incidence of dermatophytes especially *Microsporum gypseum*; *Prac. Lek.* **23** 73–76

Simordova M and Hejtmanek M 1970 Dermatophytes and other keratinophytic fungi in surface and waste waters; *Mykosen* **13** 467–471

Staib F and Evangelinos P 1968 The occurrence of geophilic dermatophytes in the region of Wuerzburg. Zeutralbl Bakterial Parasetenk Infektionskrankh Hyg. Abt. I Orig. **207** 528–540

Stepanischcheva Z G and Malkina A Y 1971 Concerning the epidemiological importance of soil as a source of mycosis infection; *Mykosen* **14** 339–401

Sur B and Ghosh G R 1980 Keratinophilic fungi from Orissa, India. I. Isolation from soils; *Sabouraudia* **18** 269–274

Toma A 1929 Sur l'infection des cheveure *in vitro* par les champignons des teignes; *Ann. Dermatol. Syph.* **10** 641–643

Vanbreuseghem R 1952 Technique biologique pour l isolement des dermatophytes du sol; *Ann. Soc. Belge, Med. Trop.* **32** 173–178

Vanbreuseghem R 1970 Geographic distribution of dermatophytes; *Int. J. Dermatol.* **9** 102–109

Volz, Paul A 1975 The keratinophilic fungi of Taiwan; *Taiwania* **20** 23–31

Wilkinson V and Lucas R L 1969 Effects of herbicides on the growth of soil fungi; *The New Phytol.* **68** 709–719

Williams J I and Pugh G J F 1974 Fungal biological flora *Chrysosporium pannorum* (Link) Hughes; *Int. Biodeterior. Bull.* **10** 75–80

Wyss O, Ludwig B J and Joiner R R 1945 The fungistatic and fungicidal action of fatty acids and related compounds; *Arch. Biochem.* **7** 415–425

Ziegler H 1966 Ektoenzyme der dermatemyzeten. II; *Mitt. Arch. Klin. Exp. Dermatol.* **226** 282–299

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 165–173.
© Printed in India.

Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model)

GILBERT TURIAN

Laboratoire de Microbiologie générale, Université de Genève, 1211 Genève 4, Switzerland

Abstract. Pregerminative polarization of the cytoplasmic content of a spore following its awakening by water imbibition is ultrastructurally featured by a 2-step sequential process: (i) general partition into a vacuole-enriched pole endowed with increased turgor pressure opposite to an energized pole of clustered mitochondria; such mitochondrial positioning would predetermine the bulging site of the germ tube by optimizing the chance event of (ii) physical contact between that plasma membrane site with the membrane of a few of the most peripherically located mitochondria of the cluster. In such a membrane interaction, the plasma membrane would presumably be electrically depolarized by mitochondrial protons vectorially extruded towards its negatively charged inside face acting as proton sink. That primary spatio-physical event would set off a train of events, the first being a local acidification of the thereby gelified cytosol excluding mitochondria while attracting vesicles into this prospective hyphal tip.

It is further suggested that the primary axiation of the germinative conidium can be controlled by slight changes in the peripheral O_2 tension guiding the mitochondrial cluster towards the elected membrane site of germ tube outgrowth.

Keywords. Polarity; fungal spores; germination; monilioid conidia.

1. Introduction

Fungal spores, and spores in general (Actinomycetes, Bacteria, etc.) are resting and resistant cellular structures. Ascospores are known as the most typical dormant cells which must be activated (heat—chemical activation) to germinate while most conidia are considered as semi-dormant (Sussman and Halvorson 1966). Such are the so-called conidia of *Neurospora* (*N. crassa, N. sitophila, N. tetrasperma*) designating the most general multinucleate, macroconidial type, their uninucleate, microconidial counterpart being much less widespread and still little known among phialoconidial types of asexual spores (Subramanian 1971; Rossier *et al* 1977). Ontogenetically, macroconidia of *Neurospora* sp. correspond to a blasto-arthroconidial type of spores (Turian and Bianchi 1972) which is that of the monilioid conidia of the form-genus *Monilia* (Turian 1976) common to the asexual stage of both *Neurospora* and *Sclerotinia* (*Stromatinia*) genera.

Water imbibition has been considered as the first elicitor of spore germination (Gottlieb 1978). That would lead first to a purely isometric physical swelling of the generally elongated spore followed by isometric growth processes (Yanagita 1957). This second growth phase is more or less developed. It is "minimized" in spores which can germinate in simple water as exemplified by the case of *Botrytis cinerea* and *Cunninghamella* sp. (Hawker 1966) as well as *Monilia* (*Sclerotinia*) *fructigena* (Najim 1979). Directly transferred from rotting fruit such as apples covered with fresh conidial rings to drops of distilled water, conidia of *Monilia fructigena* outgrow germ tubes with an efficiency of close to 100% in 2 hr at 20°C. From their "*in situ*", dry state to the water-imbibed state, they only slightly enlarge on their transverse axis. "Swelling" is

maximal in conidia of *Aspergillus* spp. (*A. niger*, *A. oryzae*, etc.) which present the most complex requirement such as amino acids (α-alanine, etc.) for their germination (Hawker 1966; Smith *et al* 1976).

Conidia (macroconidia) of *N. crassa* are intermediate in that process of isometric enlargement (2 times increased diameters), requiring single carbon (glucose, etc.) and nitrogen (NH_4NO_3 or KNO_3) sources as well as salts (Farach *et al* 1979) among which K^+ ions appear crucial (Turian *et al* 1984) for efficient outgrowth.

The sequence of structural-functional events leading from the "dry" to the outgrowing conidium is especially well-illustrated by the *Neurospora* model.

2. Dry (semi-dormant) to wet ("swollen") spore

The mature and for sometime semi-dormant conidium is one of the 8–12 units disarticulated from a macroconidial chain centrifugally budded at least 8–10 days before, at 25°C, at the tips of aerial hyphae functioning as conidiophores on the surface of a solid synthetic medium. Such a conidium can remain semidormant without loosing too much of its germinative capacity for 10–20 more days but only 10 % of the mature conidia can still germinate after 30 days maintenance on the surface of conidial cultures at 25°C (Schmit and Brody 1976).

"Dry" conidia, ultrastructurally well illustrated (figure 1, Ton-That and Turian 1978), are surrounded by a relatively thick wall covered by a thin hydrophobic sheath of lipoproteinaceous material (Turian 1966; Schmit and Brody 1976). Internally, their diffuse cytoplasm with spread free ribosomes shows a relatively poorly developed endomembrane system and uniformly distributed vacuoles and lipidic inclusions. Homogenously distributed mitochondria show a swollen electron-translucent matrix surrounding stacks of thin cristae (Weiss and Turian 1966). Such "dry" conidia are low in H_2O content (5·1 % dry weight), while their walls are richer (27 %), as well as protein and lipids (25·5 and 17·7 % dry weight, respectively), according to Owens *et al* (1958, in Gottlieb 1978). The content in polyribosomes is very low in isolated conidia (Bonnen and Brambl 1983) and not more than 10 % in quickly wet analysed conidia (Grange and Turian 1981). Energetically, such "dry" conidia have a maintenance metabolism still requiring some ATP synthesis as shown by the P/O value of 1·2–1·5 measured at this stage (Ortega Perez *et al* 1981). This value is in contrast with the close to O phosphorylation previously measured in less protective technical conditions of mitochondrial isolation (Greenawalt *et al* 1972). As for their respiratory status, such conidia have been claimed to rely essentially on the cyanosensitive cytochromic pathway (Slayman and others, see Schmit and Brody 1976). Through that pathway, there might occur a maintenance respiration slowly consuming lipids (Bianchi and Turian 1967) in addition to carbohydrate reserves, essentially trehalose (Sussman 1976).

Internal and surface changes soon follow wetting of conidia by their transfer into H_2O. The hydrophobic peripheral layer fades and disappears from the surface of water centrifuged conidia when prepared for electron microscopy. Hydration of the cytoplasmic proteins and increased volume of the vacuolar system should contribute to some initial physical swelling of conidia. Among the first biochemical events intervening already after only 5 minutes are, as summarized by Brody (1981): a sudden increase of the reducing power as probably one of the most biochemically significant

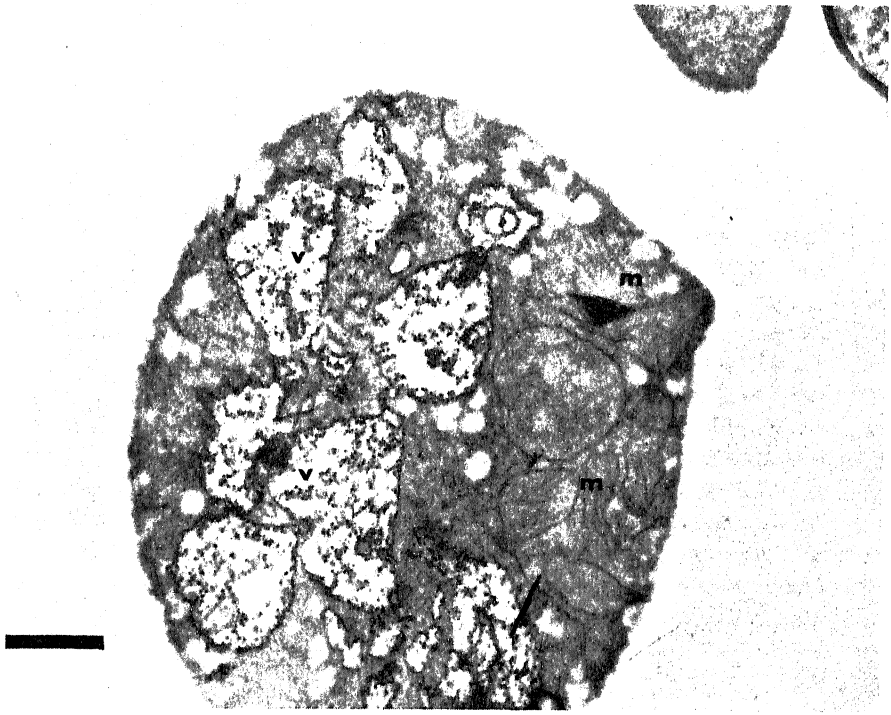


Figure 1. Ultrathin section of a pregerminating macroconidium of *N. crassa* incubated for 1 hr in liquid synthetic medium (sucrose—KNO_3—salts). Primary polarized partitioning between vacuole (v)-rich cytoplasm (left) and cluster of mitochondria(m) positioned below the plasmalemmic site bulging into the germ tube (right). Fixation in glutaraldehyde (6%), postfixation in OsO_4 (2%), staining of the thin section with uranyl-acetate before electron microphotography with the Hitachi HS-7S by N. Oulevey (Bar = 1 μm).

early events by oxidation of the glutamate reserve as the main provider of NAD(P) → NAD(P)H_2 reduction. In a comparison of dry harvested (in fluorocarbon) and wet harvested (30 minutes separative manipulation in aqueous medium), we measured a sharp increase in cytochrome oxidase and citrate synthetase activities (Ortega Perez and Turian unpublished experiments, 1978) paralleling an increased O_2 consumption. The cytochrome oxidase complex is known to be already assembled in the conidia of *N. crassa*, a situation in contrast to that occurring in conidia of *Botryodiplodia theobromae* (Brambl 1981). Contrarily to dormant spores of *Botryodiplodia theobromae*, conidia of *N. crassa* preserve all the components necessary for a functional electron transport chain. These are responsible for the increased respiration rate that begins immediately after the conidia are suspended in an incubation medium (Stade and Brambl 1981). Ribosomes massively assemble into polysomes in hydrated conidia (Mirkes 1974; Grange *et al* 1983) thereby allowing active protein synthesis in the cytoplasm while conferring cycloheximide sensitivity to the germinative process (Van Etten *et al* 1983).

What from the outside is viewed as a first germinative stage of more or less expanded isotropic growth is now paradoxally appearing when studied inside as providing the

aleatory segregation of cell constituents determining the preparatory cytoplasmic axiation toward the prospective site of germ tube outgrowth. Axis orientation can be predetermined in spores equipped from the start with a germ pore, while in those deprived of it, as in monilioid conidia placed in an isotropic medium, it can only result from an aleatory distribution of the organelles. Their orientation can however be directed by outside factors as exemplified by chemotropic reactions to physico-chemical gradients (O_2, CO_2, organic compounds, etc.) or unilateral physical signals (light, etc.).

In conidia of *N. crassa* incubated for 1–2 hr in liquid nutritive media, there intervenes an internal partitioning of the initially relatively homogenously distributed spore content. This implies polar shifting of the vacuolar system and opposite polar clustering of many mitochondria which could generally be observed by the end of the isometric growth stage (figure 1). Such incipient partitioning was also observed in conidia of *Monilia fructigena* in which the initially homogeneous cytoplasmic content vacuolizes at the opposite pole of the prospective site of outgrowth. The vacuoles then move to the central, wide portion of the conidium in preparation for the frequent emergence of a second germ tube of opposite polarity (bipolar germination, figure 2a). We have quite recently observed that the polar clustering of mitochondria leads to the contact of a few of these organelles with the plasmalemma (Turian and Geissler 1984) and hypothesized that such transient contact could locally depolarize the peripheral membrane at the prospective outgrowth site of the germ tube. However, such asymmetric events must necessarily be discrete because of the well-known "in vivo" mobility of mitochondria and could only be observed in a "lucky section" or favored in its study by serial sections of a "swollen" conidium (Turian and Geissler 1984). They should lead to the vectorial extrusion of protons described elsewhere (Turian 1983a) and the acidification produced by local gelification of the cytosol would push back the mitochondria thereby creating the so-called exclusion zone of the apex in elongation (see below).

3. "Swelling" to outgrowth stage

Relative deacidification of the more or less "swollen" sporal cytoplasm appears to be required as a preparatory step to transition from the "swelling" to the outgrowth stage. In conidia of *N. crassa*, it would be the role of exogenous K^+ ions intruded in exchange for H^+ accumulated in the relatively more acid cytosol of dormant conidia (Turian *et al* 1984). Such excess protons could result from at least partial P/O uncoupling in mitochondria of such spores (Schmit and Brody 1976; Ortega Perez *et al* 1981) as well as from the accumulation of organic acids such as succinic and malic acids (Owens *et al* 1958). Such increased acidity of the cytosol could be responsible of its gelification preventing cytoplasmic streaming as observed by the non-mobility of organelles in dormant spores (see Gregory 1966). The secondary increase of pH in the cytoplasm of pre-emergence spores might be, by analogy with animal embryos (Grainger *et al* 1979), the necessary condition for DNA synthesis in G_1 phase conidial nuclei (Loo 1976) as a preparative step for the first nuclear mitosis microscopically detected after the outgrowth of the germ tube. Relative cytoplasmic alkalinization might be also considered as preparatory to the subsequent polarized outgrowth; this is suggested in the "emergency" conditions when nitrate is provided as single N source and

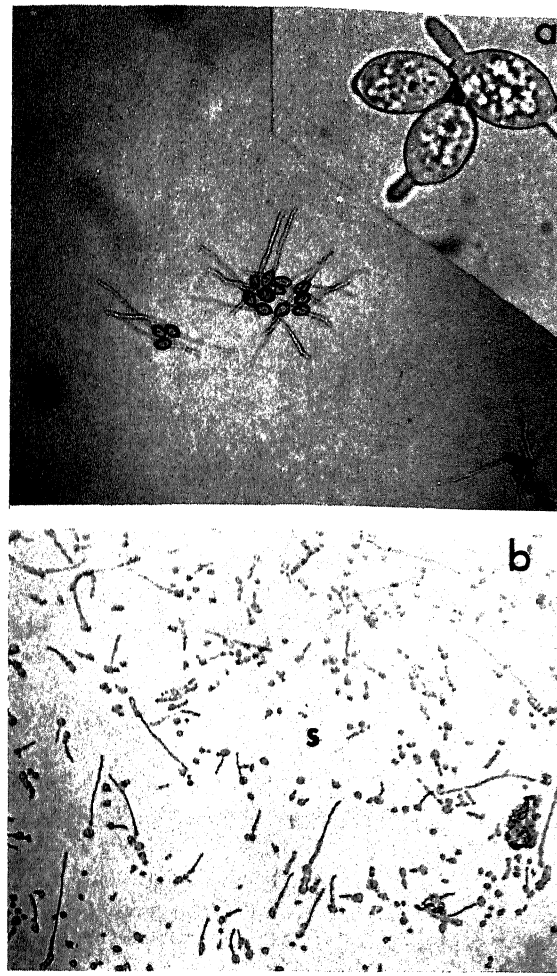


Figure 2. **a.** Negative autotropism of germ tubes of conidia of *Monilia fructigena* elongating in distilled water (20°C) spread below the coverslip on a glass slide in a wet chamber (×100). Insert: bipolar outgrowing conidium of *Monilia fructigena* in distilled water: central positioning of the vacuolar system (×250). **b.** Germ tubes outgrowing from conidia of *N. crassa* towards the central open space (s) in a drop of liquid synthetic medium (sucrose—NH_4NO_3—salts) poured on a glass slide in a wet chamber at 20°C (×50).

germination could even be observed with $NaNO_3$ (even though delayed compared to KNO_3, Löffel and Turian, unpublished observations, 1982). In this case, it may be supposed that Na^+ ions copenetrate with NO_3^- anions into the sporal cytoplasm in exchange for the excess internal protons, thereby providing the necessary deacidification cations. In the presence of the "dual offer" of NH_4NO_3, it could be inferred that in those conditions of preferential uptake of NH_4^+, only the conducting channels for the smaller K^+ ions are opened for exchange with effluxed H^+. The NH_4^+ actively participates in the amination of organic acids possibly acting as an internal sink which should contribute to the efficiency of H^+ exchange and, by consequence, of

germination in an ammonium source when compared to NO_3^- provided alone (Guignard *et al* 1984).

When local reacidification intervenes at the site of presumptive germ tube outgrowth, both by vectorial extrusion of protons from "contact mitochondria" and by reintrusion of exogenous H^+ (see below), K^+ should be extruded at that site to insure such an acidification process. With pH-indicators (Turian 1980) and fluorescent probes (Turian 1983b), we could locate the acidified site of germ tube outgrowth already at the end of the "swollen" stage. Using the sophisticated technique of vibrating electrode, Kropf *et al* (1983) have detected a localized increase in the internal pH corresponding to the entrance point of an exogenous proton current and predicting the site of lateral hyphal branching.

Our question is now, what is the inceptive event at the site of such an "acid cytosolic spot" or of such a proton current entry?

4. Outgrowth

We have proposed the hypothesis of the aleatory back-positioning of mitochondria allowing vectorial extrusion of protons towards the prospective emerging germ tube (Turian 1980). Thereby, we have considered the mitochondria as creators of the endogenous heterogeneity necessary to locate the emergence site. We present below a few arguments in favour of our so-called endogenous hypothesis while attempting to reconcile it with its exogenous bioelectrical alternative (Harold 1982). In this new view, the unipolar acidic spot detected with pH indicators and fluorescent probes is considered to be the visible marker of the presumptive outgrowth site (Turian 1980, 1983b), but secondary to the initial depolarizing contact of the few mitochondria with the plasma membrane. In fact, when an acidic spot could be microscopically localized, the "contact mitochondria" must have already retreated from the front membrane because of the backward extension of the presumably gelified acidic cytosol (+charged) attracting wall vesicles (−charged) and thereby becoming the so-called exclusion zone of the germ tube apex in formation. Such auto-exclusion of the mitochondria from the expanding apical cytosolic zone following its presumed gelification has been ascribed to a type of vectorial extrusion of protons from polarly uncoupled mitochondria (Turian 1980, 1981). The acidified cytosol segregated from the frontal cluster of mitochondria below the apical plasmalemma would function as a proton sink self-sustained and amplified by the convergence towards it of hundreds of vesicles: among these there would be (a) microvesicles associated with microfilaments as filasomes of the apical aggregate (Hoch and Howard 1980) of the acid and reductive "Spitzenkörper" (Turian 1983a; Gooday 1983); (b) the larger vesicles presumably containing, as in algal apices (Quatrano 1974), anionic polysaccharidic wall precursors.

Cytoplasmic Ca^{2+} levels, crucial for regulation of exocytosis of germ tube apical vesicles, must be controlled by Ca^{2+} removal systems, including pumps and antiporters. When back-retreated from the young tip, mitochondria could play that role in sequestering Ca^{2+} ions contributing to some degree of uncoupling by dissipation of the proton gradient from such apically positioned mitochondria (Turian 1979). This explanation would, however, not fit well with the action of Ca^{2+} translocation dependent upon ATP (Ca^{2+}-ATPase). Rather, there could parallely be

antiport activity between reintruding protons and expelling of Ca^{2+} ions (and K^+, see above) at the ultimate elongating apex.

Besides growth factors there should also be activity of guidance factors in the elongation phase of the outgrowing germ tube. In *N. crassa*, we have observed a chemotropic reaction of the germ tubes towards the central zone of the liquid drop of synthetic medium on a slide placed in a wet chamber (figure 2b) as if germ tubes were outgrowing and elongating towards the open space meaning more dissolved O_2, as was also suggested for the oriented rhizoids of *Blastocladiella* (see Gooday 1983). The orientation of the conidial germ tubes towards the center of the liquid drop where practically no conidia were present, can therefore be considered as negative autotropism to open space, with the conidia outgrowing away from their close neighbors, either towards oxygen or away from stalling factors as proposed by Gooday (1983) about oriented growth of *Blastocladiella* thalli. It is in fact well-known that many fungal spores, when placed in groups, germinate so that their tubes grow away from each other (Robinson 1973a). Such divergently oriented growth or autotropism can be interpreted as a positive chemotropism towards an optimum oxygen concentration (Robinson 1973b). In *Monilia fructigena* the autotropic reaction of sprouted germ tubes is also negative (figure 2a).

For both these cases the election site of the germ tube is situated towards the exterior where more O_2 should be available to the conidial cells; that locally increased O_2 concentration might contribute to the attraction of mitochondria towards the external membrane. The actively O_2-consuming mitochondria—especially those apically located and presumably partially uncoupled—could further contribute to the guidance of the elongating germ tube towards the open, O_2-richer liquid space as illustrated by our microscopical images of star-like figures of hyphal filaments.

Our new views about the polarizing processes in "budding" conidia lead us also to think that the proposed polarizing effect of the nuclear spindle pole body oriented towards the prospective budding site of the yeast (Byers and Goetsch 1975) results from a secondary orientation of the nucleus and its mitotic apparatus along the axis of the previously developed acidic gradient. This opinion is supported by the fact that macroconidia of *Neurospora* contain from 2–5 nuclei but with most generally only one outgrown germ tube from the mitochondria-clustered zone. From conidia of *Monilia fructigena*, there are generally two germ tubes, which emerge from two opposite poles, each with its mitochondrial cluster separated by the largest vacuoles (figure 2a). One among the numerous ultrathin sections realized by Najim (1979) has been luckily cut through a nucleus bearing its yet undivided spindle pole body on the opposite side from the outgrowing germ tube.

5. Conclusions

The onset of sporal polarity is a 2-step process progressing in the following order: (i) random (isotropic medium) or induced (anisotropic conditions) partition of the cytoplasmic content of the mother cell. Osmotic effects should intervene in the initial increase of the cytoplasmic turgor pressure resulting from an activation at the water imbibition phase of hydrolysis of reserve products as well as increased ions exchange (K^+, Ca^{2+}). The asymmetrical clustering of mitochondria below a given site of the plasmalemma would predetermine the pole of germ tube outgrowth because of the increased chance of (ii) local depolarization of the plasmalemma by vectorially-

extruded protons from "contact mitochondria" leading to segregation of acid, positively-charged and gelified cytosol attracting negatively-charged vesicles at the elected outgrowth site.

References

Bianchi D E and Turian G 1967 Lipid content of conidia of *Neurospora crassa; Nature (London)* **214** 1344–1345

Bonnen A and Brambl R 1983 Germination physiology of *Neurospora crassa* conidia; *Exp. Mycol.* **7** 197–207

Brambl R 1981 Respiration and mitochondrial biogenesis during fungal spore germination, in *The fungal spore: morphogenetic controls* (eds) G Turian and H R Hohl (London: Academic Press) pp. 585–604

Brody S 1981 Genetic and biochemical studies on *Neurospora* conidia germination and formation, in *The fungal spore: morphogenetic controls* (eds) G Turian and H R Hohl (London: Academic Press) pp. 605–626

Byers B and Goetsch L 1975 Behaviour of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*; *J. Bacteriol.* **124** 511–523

Farach M C, Farach H and Mirkes P E 1979 Control of development in *Neurospora crassa*: nutrient requirements for conidial germ tube emergence, elongation and metabolic activation; *Exp. Mycol.* **3** 240–248

Gooday G W 1983 The hyphal tip, in *Fungal differentiation* (ed.) J E Smith (New York: Marcel Dekker Inc.) pp. 315–356

Gottlieb D 1978 *The germination of fungus spores* (ed.) J G Cook (Durham, England: Meadowfield Press)

Grainger J L, Winkler M M, Schen S S and Steinhardt R A 1979 Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo; *Dev. Biol.* **68** 396–406

Grange F and Turian G 1981 Polysomes in dry- and wet-harvested ungerminated conidia of *Neurospora crassa*; *FEMS Microbiol. Lett.* **12** 283–285

Grange F, Ojha M and Turian G 1983 Changes in protein synthesis in heat-treated and normally germinating conidia of *Neurospora crassa; Experientia* **39** 1111–1112

Greenawalt J W, Beck D P and Hawley E S 1972 Chemical and biochemical changes in mitochondria during morphogenetic development of *Neurospora crassa*, in *Biochemistry and biophysics of mitochondrial membranes* (eds) J F Azzone and A P Carafoli (New York: Academic Press) pp. 541–558

Gregory P H 1966 The fungus spore: what it is and what it does, in *The fungus spore* (ed.) M F Madelin (London: Butterworths) pp. 1–14

Guignard R, Grange F and Turian G 1984 Microcycle conidiation induced by partial nitrogen deprivation in *Neurospora crassa; Can. J. Microbiol.* **30** 1210–1215

Harold F M 1982 Pumps and currents: A biological perspective; *Curr. Top. Membr. Transp.* **26** 485–516

Hawker L E 1966 Germination: morphological and anatomical changes, in *The fungus spore* (ed.) M F Madelin (London: Butterworths) pp. 151–162

Hoch H C and Howard R J 1980 Ultrastructure of freeze-substituted hyphae of the basidiomycete *Laetisaria arvalis; Protoplasma* **103** 281–297

Kropf D L, Lupa M D A, Caldwell J H and Harold F M 1983 Cell polarity: endogenous ion currents precede and predict branching in the water mold *Achlya; Science* **220** 1385–1387

Loo M 1976 Some required events in conidial germination of *Neurospora crassa; Dev. Biol.* **54** 201–213

Mirkes P E 1974 Polysomes, ribonucleic acid, and protein synthesis during germination of *Neurospora crassa* conidia; *J. Bacteriol.* **117** 196–202

Najim L 1979 *Contrôle morphogénétique et étude ultrastructurale de Monilia fructigena (Ader et Ruhl)*. Thèse No 1926, Université de Genève

Ortega Perez R, Michéa-Hamzehpour M and Turian G 1981 Respiratory properties of mitochondria isolated from conidia of *Neurospora crassa; J. Gen. Microbiol.* **127** 309–314

Owens *et al* 1958 see Gottlieb D 1978

Quatrano R S 1974 Development of cell polarity, in *Experimental marine biology* (ed.) R Marisca (New York: Academic Press) pp. 303–346

Robinson P M 1973a Autotropism in fungal spores and hyphae; *Bot. Rev.* **39** 367–384

Robinson P M 1973b Oxygen-positive chemotropic factor for fungi?; *New Phytol.* **72** 1349–1356

Rossier C, Ton-That T C and Turian G 1977 Microcycle microconidiation in *Neurospora crassa; Exp. Mycol.* **1** 52–62

Schmit J C and Brody S 1976 Biochemical genetics of *Neurospora crassa* conidial germination; *Bacteriol. Rev.* **40** 1–41

Smith J E, Gull K, Anderson J G and Deans S G 1976 Organelle changes during fungal spore germination, in *The fungal spore, form and function* (eds) D J Weber and W M Hess (New York: John Wiley and Sons) pp. 301–354

Stade S and Brambl R 1981 Mitochondrial biogenesis during fungal spore germination: respiration and cytochrome c oxidase in *Neurospora crassa; J. Bacteriol.* **147** 757–767

Subramanian C V 1971 The phialide, in *Taxonomy of fungi imperfecti* (ed.) B Kendrick (Toronto: University of Toronto Press) pp. 92–119

Sussman A S 1976 Activators of fungal spore germination, in *The fungal spore, form and function* (eds) D J Weber and W M Hess (New York: John Wiley and Sons) pp. 101–139

Sussman A S and Halverson H O 1966 *Spores: Their dormancy and germination* (New York and London: Harper and Row)

Ton-That T C and Turian G 1978 Ultrastructural study of microcyclic macroconidiation in *Neurospora crassa; Arch. Microbiol.* **116** 279–288

Turian G 1966 The genesis of macroconidia of *Neurospora*, in *The fungus spore* (ed.) M F Madelin (London: Butterworths) pp. 61–66

Turian G 1976 Spores in Ascomycetes, their controlled differentiation, in *The fungal spore, form and function* (eds) D J Weber and W M Hess (New York: John Wiley and Sons) pp. 715–788

Turian G 1979 Cytochemical gradients and mitochondrial exclusion in the apices of vegetative hyphae; *Experientia* **35** 1164–1166

Turian G 1980 Germination of conidia (*Neurospora, Trichoderma*): evidence of an electrogenic sink of protons mitochondrially-extruded into emerging tubes; *Ber. Schweiz. Bot. Ges.* **90** 203–212

Turian G 1981 Low pH in fungal bud initials; *Experientia* **37** 1278–1279

Turian G 1983a Concepts of fungal differentiation, in *Fungal differentiation* (ed.) J E Smith (New York: Marcel Dekker Inc.) pp. 1–18

Turian G 1983b Polarized acidification of germ tube outgrowth from fungal spores (*Morchella* ascospores, *Neurospora* conidia); *Bot. Helv.* **93** 27–32

Turian G and Bianchi D E 1972 Conidiation in *Neurospora; Bot. Rev.* **38** 119–154

Turian G and Geissler C L 1984 Further clues for the involvement of mitochondria in the initiation of germ tube outgrowth from fungal conidia (*Neurospora* model); *Bot. Helv.* **94** 407–410

Turian G, Loeffel R and Michéa-Hamzehpour M 1984 Requirement for K^+ at conidial germination in *Neurospora crassa; Bot. Helv.* **94** 301–306

Van Etten J L, Dahlberg K A and Russo G M 1983 Fungal spore germination, in *Fungal differentiation* (ed) J E Smith (New York: Marcel Dekker Inc.) pp. 235–266

Weiss B and Turian G 1966 A study of conidiation in *Neurospora crassa; J. Gen. Microbiol.* **39** 85–94

Yanagita T 1957 Biochemical aspects of germination of conidiospores of *Aspergillus niger; Arch. Mikrobiol.* **26** 329–344

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 175–196.
© Printed in India.

Enzymes from thermophilic fungi: Proteases and lipases

BHAVDISH N JOHRI, SANJAY JAIN and SATISH CHOUHAN

Department of Microbiology, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India

Abstract. Microbial enzymes of thermophilic fungi offer several advantages since they are highly thermostable and also have minimum chances of fermenter contamination at elevated temperatures. In this review, we have focussed on the proteases and lipases of thermophiles together, because of their wide ranging applications in the field of brewery, textile, dairy, pharmaceutical and leather industries.

Keywords. Thermophilic fungi; microbial hydrolases; proteases; lipase; microbial rennet.

1. Introduction

Microbial enzymes have been used in varied facets of food, chemical and pharmaceutical industries over the years, and major applications have also been catalogued by several workers (Davies 1963; Gutcho 1974; Reed 1975; Wiseman 1975; Abbott 1976; Underkofler 1976; Aunstrup 1977). But the last couple of decades have seen increasing attention being diverted to industrial production from thermophilic microorganisms. Among various enzymes, amylase, cellulase, lipase, protease and xylanase have been intensively investigated (Somkuti *et al* 1969; Arima *et al* 1972; Ong and Gaucher 1973, 1976; Coutts and Smith 1976; Satyanarayana 1978; Oso 1978, 1979; Flannigan and Sellars 1978; Khan *et al* 1979; Ogundero 1980; Yoshioka *et al* 1981; Olutiola 1982; Vandamme *et al* 1982; Satyanarayana and Johri 1983; Chouhan 1984).

2. Importance of thermophilic enzymes

Several reasons can be assigned to the attention that thermophilic moulds in particular have received lately. Their growth temperatures are generally much higher than ambient (45–60°C) and thus industrial processes are beset with lesser fermenter contamination and cooling costs. Furthermore, enzymatic abilities of thermophilic moulds are also much superior by way of their greater thermostability and better production under nutritional conditions on a comparative basis with mesophiles. Enzymes from thermophiles have been profitably utilized in various industries, because of their wide ranging applications as well. The production of better known enzymes by thermophilic fungi is summarized in table 1. Among other reasons for their commercialization, Ramaley (1972) reported greater resistance for six enzymes from an extreme thermophile to losses during purification than for the same enzymes from mesophilic bacteria. Most of these enzymes show much lesser losses during storage as well.

There are now indications that processes based on thermophiles can offer potential for lower capital and operating costs in some fermentations. The cost savings would be those associated with the installation, maintenance and operation of heat exchange

Table 1. Enzymes produced by thermophilic fungi.

Organism	Enzymes	References
Aspergillus fumigatus	AM, C, X, LIP	Satyanarayana (1978), Vandamme *et al* (1982), Flanningan and Sellars (1978), Satyanarayana and Johri (1981)
Acremonium alabamensis	X	Satyanarayana and Johri (1983)
Achaetomium macrosporum	AM	Satyanarayana (1978)
Absidia corymbifera	LIP	Satyanarayana and Johri (1981)
Chaetomium thermophile	LIP, X, C, AM	Satyanarayana and Johri (1981), Satyanarayana and Johri (1983), Satyanarayana (1978), Ogundero (1979)
C. thermophilie var. *coprophile*	B-G	Luis and Becker (1973)
C. thermophile var. *dissitum*	C	Eriksen and Goksoyr (1976)
Crysosporium pruinosum	LC	Rosenberg (1978)
Humicola grisea	LIP	Satyanarayana (1978)
H. grisea var. *thermoidea*	LIP, U, D, R, C	Adams and Deploey (1978), Ogundero (1980), Fergus (1969)
H. insolens	C, D, LIP, R, U, AM	Ramabadran (1969), Adams and Deploey (1978), Ogundero (1979), Jain *et al* (1979)
H. insolens YH-8	X, C	Yoshioka *et al* (1981), Hayashida and Yoshioka (1980)
H. lanuginosa	LIP, X, GA, T, I, AM, AMG, C	Liu *et al* (1972, 1973a), Satyanarayana and Johri (1981, 1983), Rao and Shastri (1981), Rao and Maheshwari (1979), Prasad and Maheshwari (1979), Maheshwari *et al* (1983), Satyanarayana (1978), Tayler *et al* (1978), Olutiola (1982).
Malbranchea pulchella var. *sulfurea*	C, AM, LIP, X	Premabai and Narasimha Rao (1963), Ogundero (1979), Jain *et al* (1979)
Mucor miehei	C, LIP, U, D, AM	Somkuti (1974), Adams and Deploey (1976, 1978), Deploey *et al* (1981), Jain *et al* (1979)
M. pusillus	AM, C, LIP	Somkuti and Steinberg (1980), Somkuti *et al* (1969), Somkuti and Babel (1968a), Satyanarayana and Johri (1981), Jain *et al* (1979), Ogundero (1980)

Myceliophthora thermophila D-14	C	Sen *et al* (1981)
M. thermophila	C	Chakrabarty and Sen (1982)
Papulaspora thermophila	C, AM, D, LIP, U	Chapman *et al* (1975), Adams and Deploey (1978)
Sporotrichum thermophile	LIP, U, D, C, X, B-G	Adams and Deploey (1978), Satyanarayana and Johri (1981), Coutts and Smith (1976) Margaritis *et al* (1983), Singh (1982), Satyanarayana and Johri (1983), Canevascini and Meyer (1979)
S. pulverulentum	C, LC	Streamer *et al* (1975), Rosenberg (1978)
Stibella thermophila	CMC	Fergus (1969)
Talaromyces bassochlamydoides YH-50	X	Yoshioka *et al* (1981)
T. dupontii	AM, C	Jain *et al* (1979), Ogundero (1979)
T. emersonii	C, AMLIP	Oso (1978), Coughlan and Hale (1981), Oso (1979)
Talaromyces sp.	C, X	Nishio *et al* (1981), Satyanarayana and Johri (1983)
T. thermophilus	LIP	Ogundero (1980)
Thermoascus aurantiacus	C, X, AM, LIP	Tong and Cole (1982), Satyanarayana and Johri (1983), Jain *et al* (1979), Ogundero (1979), Ogundero (1980)
T. crustaceus	AM, C.	Ogundero (1979)
Thielavia minor	LIP	Satyanarayana and Johri (1981)
T. sepedonium	CMC	Flanningan and Sellars (1972)
T. thermophila	C	Rosenberg (1978)
Torula thermophila	LIP, D, R, U, AM, AMG, C	Adams and Deploey (1978), Satyanarayana (1978), Subrahmanyam *et al* (1977), Satyanarayana and Johri (1983), Jain *et al* (1979)

AM = Amylase, B-G = β-Glucosidase, C = Cellulase, LIP = Lipase, D = DNAase, R = RNAase,
U = Urease, X = Xylanase, AMG = Amyloglucosidase, I = Invertase, T = Trehalase,
CMC = Carboxymethyl cellulase, LC = Lignocellulase, GA = Glucoamylase.

equipment normally required for the removal of heat produced in large scale mesophilic fermentations.

To above considerations can be added the following specific properties which make their applications in enzyme engineering truly attractive:

(a) They allow enzyme reactors to be operated at higher temperatures resulting in such advantages as decreased fluid viscosity and increased solute solubility. There is also a potential advantage of increased reaction rates.
(b) Some enzymes from thermophiles exhibit extraordinary stability in the presence of detergents and aqueous-organic solvent solutions (Endo 1962; Ohta 1967). This may be of value if mixed solvent systems are to be used to improve the solubility of substrates and products and thus this allows greater productivity in an enzyme reactor.
(c) Heat stability and solvent resistance of these enzymes may prevent denaturation during chemical coupling procedures. This will tend to provide higher activities of the immobilized enzyme which in turn increases the operating life of an immobilized enzyme reactor.
(d) A major problem in operating an enzyme reactor at room temperature is the continuous potential for microbial contamination. By operating such systems at higher temperatures, this problem can be easily eliminated to a large extent.

3. Why proteases and lipases?

In this review, the proteases and lipases of thermophiles have been put together, because of their extensive use in several industries like dairy industry etc.

Investigations in which microbial enzyme preparations were introduced include cheese curds to speed and influence flavour and texture qualities (Iwasaki *et al* 1967; Somkuti and Babel 1968a, b; Iwasaki and Kosikowski 1973; Kosikowski and Iwasaki 1975; Nakanishi and Itoh 1974; Arnold *et al* 1975; Sood and Kosikowski 1979). Some enzyme preparations (protease and lipase) intended for this purpose have also been patented (Somkuti and Babel 1968a, b; Somkuti *et al* 1969; Arima *et al* 1972). The presence of lipase activity in microbial protease preparations (Richardson *et al* 1967; Somkuti *et al* 1969) results in acceleration of flavour development in some types of cheeses, since it is known that the characteristic flavour of several cheeses results from the hydrolysis of butterfat during curing. However, extensive hydrolytic breakdown of butterfat with concomitant release of low mol. wt. fatty acids may result in development of undesired (rancid) flavours.

Iwasaki and Kosikowski (1973) and Kosikowski and Iwasaki (1975) have screened a large number of microbial enzymes including protease and lipase, for their contribution towards acceleration of cheese flavour. Moreover, in recent years microbial rennets have been found to be acceptable substitute for calf rennet and thermophilic fungi have found an important place in the manufacturing processes of these enzymes. Strains of *Mucor pusillus* and *Mucor miehei* have been reported to produce milk clotting enzymes on commercial scale and these have been used as suitable substitute for calf rennin (Arima *et al* 1967; Iwasaki *et al* 1967; Richardson *et al* 1967; Somkuti and Babel 1967; Yu *et al* 1969; Charles *et al* 1970; Prins and Nidson 1970; Sternberg 1972, 1976). Extensive production of a wide variety of cheeses *e.g.*, Cheddar, Swiss, Italian, etc. with partially purified preparations of fungal rennin also has been judged to be equal, or superior, to those obtained with animal rennet.

4. Proteases

Proteases are enzymes hydrolyzing the peptide links of proteins or peptides. They are divided into endoenzymes or endo-peptidases promoting attack within the chain of macromolecules and exoenzymes or exopeptidases hydrolyzing terminal amino acids (Neurath 1957).

4.1 *Classification*

The class exoenzyme includes α-amino acyl-peptide hydrolases (EC 3.4.1) and peptidyl amino acid hydrolases (EC 3.4.2) both splitting off terminal amino acids, the former embracing aminopeptidases and the latter carboxypeptidases. A separate class, dipeptide hydrolases, (EC 3.4.3) splits dipeptides and the endoenzyme, peptidyl peptide hydrolases (EC 3.4.4) acts on peptide links within the chain; familiar animal proteases such as pepsin and trypsin are in this group, as also aspergillopeptidase A (EC 3.4.4.17) which attacks bonds involving the carboxyl group of arginine or leucine.

Microbial proteases have been classified in various ways, but the most satisfactory scheme appears to be one based on pH optima and inhibitor sensitivity (Keay 1971; Ong and Gaucher 1973) (table 2). Based on the mechanism of catalytic action, microbial proteases are characterised as (i) serine proteases (ii) thiol proteases (iii) metal proteases, and (iv) acid proteases. Morihara (1974) has further classified proteases according to their side chain specificity, on the assumption that it is a more acceptable basis than biological activity.

4.2 *Application*

Microbial proteases have wide ranging applications in the field of brewery, dairy, textile and leather industries. One of the largest use of microbial proteases is, however, in the baking industry. The glutten proteins of the flour are responsible for the unique and variable properties of bakery doughs.

Fungal proteases find applications mainly in food industry and this is particularly true of acid proteases which among other functions can substitute in the activities associated with papain, rennin and pepsin. *Aspergillus* and *Mucor* species are the most important source of fungal proteases in industry; *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Mucor pusillus*, *Mucor miehei* and *Rhizopus* species are used in preparation of oriental foods such as tempeh and koji and in cheese making as a substitute for natural rennet; others such as *M. pusillus*, *M. miehei*, *Penicillium roqueforti* and *P. camemberti* are important sources of milk clotting enzymes (Arima *et al* 1967; Sternberg 1976). The protease from *Endothia parasitica* is used in cheese manufacture (Sardinas 1968). Some important applications of proteases have been summarised in table 3.

4.3 *Production and regulation*

Many mesophilic fungi have been exploited for the release of proteolytic enzymes (Dion 1950; Johnson and Martin 1964; Singh and Martin 1960; Oleniacz and Pisano 1968; Wang and Hesseltine 1965; Mukherjee and Chaudhari 1977; Patil and Shastri 1981). Among thermophilic fungi, species of *Achaetomium*, *Chaetomium*, *Humicola*, *Mucor*, *Torula*, *Malbranchea*, *Penicillium*, *Rhizopus*, *Sporotrichum*, and *Talaromyces* are

Table 2. Properties of microbial extracellular proteases.

Type	Source	pH range		Mol. wt. (approx.)	Metallo-enzyme	Inhibited by			Esterase activity	Main uses
		Max. activity	Max. stability			DFP	EDTA	DAN		
Acid proteases	Mainly fungi	2–5	2–6	35000	No	No	No	Yes	Limited	Cheese Industry Food Industry
Neutral proteases	Bacteria and fungi	7–8	7–9	45000	Yes	No	Yes	No	Absent	Food processing
Alkaline proteases	Bacteria and fungi	9–11	5–10	27000	No	Yes	No	No	Very high	Detergent additive Leather tanning

DFP: diisopropyl phospho fluoriodate, EDTA: ethylene diamine tetra acetic acid, DAN: diazoacetyl nor leucine methyl ester, (Fogarty and Kally.)

known to possess good protease(s) activity. The extracellular protease production by thermophilic moulds has been studied by several workers (Ramabadran 1970; Ong and Gaucher 1973, 1976; Zakirov *et al* 1975; Karavaeva *et al* 1975; Karavaeva and Mukhiddinova 1976; Khan *et al* 1979; Satyanarayana and Johri 1983). Many of these are known to produce good amount of acidic (Somkuti and Babel 1967, 1968a; Hashimoto *et al* 1972, 1973; Emi *et al* 1976; Lasure 1980; Eriksson and Petterson 1982), neutral (Hayashida and Hungo 1968) and alkaline proteases (Craveri *et al* 1974; Karavaeva *et al* 1975; Zakirov *et al* 1975; Voordouw and Roche 1975; Ong and Gaucher 1976) and milk clotting enzymes (Arima *et al* 1967, 1968; Sardinas 1972; Oka *et al* 1973; Sternberg 1976) (table 4).

Many of the studies on synthesis of microbial extracellular proteases have dealt with physiological and environmental factors affecting enzyme production. Even though there are distinct differences between organisms, synthesis of many proteases is governed, at least in part, by the concentration of available individual nutrients; in particular, catabolic repression and end product inhibition appear to be two main regulatory mechanisms governing protease synthesis.

Extracellular proteases are known to be produced in greater quantities than endoproteases (Ong and Gaucher 1973; Zakirov *et al* 1975). The extent of protease production by micro-organisms is dependent upon a variety of factors including the make up of the fermentation medium. The media used for commercial production of fungal proteases generally include raw materials of plant origin (Aunstrup 1971). Protease production by thermophilic fungi has been studied on natural proteinaceous sources *viz*, wheatbran, soyabean meal, cornmeal, casein and egg albumin (Somkuti and Babel 1967, 1968a; Hashimoto *et al* 1972, 1973; Ong and Gaucher 1973, 1976; Shenolikar and Stevenson 1982; Satyanarayana and Johri 1983; Chouhan 1984).

Protease production of *Malbranchea pulchella* var. *sulfurea* was stimulated in 2% casein while 1% casamino acids or 0·75% yeast extract completely inhibited it (Ong and

Table 3. Industrial applications of protease and lipase.

Industry	Application	Enzyme	Source
Baking and milling	Bread baking	Protease, Lipase	Fungal, Plant
Brewing	Chill proofing	Protease	Bacterial, Fungal, Plant
Cereals	Condiments	Protease	Bacterial, Fungal Plant
Dairy	Milk, protein hydrolysates,	Protease	Bacterial, Fungal
	Cheese production	Protease Lipase	Fungal, Bacterial Animal
	Evaporated milk, stabilization	Protease	Fungal
	Modifying milk fats for flavor	Lipase	Animal, Fungal
Dry cleaning	Spot removal	Protease	Bacterial, Fungal, Animal
Eggs, dried	Improving whip of albumen	Lipase	Pancreatin
Feeds, animal	Pig starter rations	Protease	Bacterial, Fungal, Animal
	Poultry rations	Protease	Bacterial, Fungal
	Cattle rations	Protease	Fungal, Bacterial
Food protein systems	Gelatin degreasing bones	Lipase	Fungal
Leather	Bating	Protease	Bacterial, Fungal
	Unhairing	Protease	Plant, Bacterial, Fungal
Meat, fish	Meat tenderizing	Protease	Fungal, Bacterial, Plant
	Tenderizing casings	Protease	Fungal, Bacterial, Plant
	Condensed fish solubles	Protease	Fungal, Bacterial, Plant
Pharmaceutical and clinical	Digestive aids	Protease	Fungal, Bacterial, Plant
		Lipase	Fungal, Pancreatin
Photography	Recovery of silver from spent film	Protease	Bacterial
Textile	Desizing of fabrics	Protease	Bacterial, Fungal, Animal
Medicine	Relief of inflammation, Bruises, Blood clot	Protease	Bacterial, Fungal, Plant
Miscellaneous	Drain cleaners and waste disposal	Lipase	Pancreatin, Fungal
	Resolution of racemic mixtures of amino acids	Protease	Fungal

Gaucher 1976). Similarly, addition of ammonium salts to wheatbran medium enhanced acid protease production of *Penicillium duponti* K-1014 while nitrate proved detrimental (Hashimoto *et al* 1972). Sulfur compounds exert a controlling effect on acid protease production of *M. pusillus* while inorganic phosphates do not inhibit enzyme synthesis (Somkuti and Babel 1967). The regulation of acid protease of *M. miehei* has been reported to be under the influence of nutritional conditions (Lasure 1980). The production of extracellular protease of thermophilic *Achaetomium macrosporum*, *Chaetomium thermophile*, *Humicola lanuginosa*, *Sporotrichum thermophile* and *Torula thermophila* has been shown to be enhanced by the addition of casein, gelatin, glucose and maltose (Satyanarayana and Johri 1983).

The production of thermostable alkaline extracellular protease has been reported

Table 4. Sources of proteases produced by thermophilic fungi.

Organism	Types	References
Aspergillus fumigatus	Extracellular acid protease	Paneerselvam *et al* (1978)
Achaetomium macrosporum	Extracellular protease	Satyanarayana and Johri (1983)
Chaetomium thermophile	Extracellular protease	Satyanarayana and Johri (1983)
C. thermophile var. *coprophile*	Extracellular alkaline protease	Ong and Gaucher (1973)
C. thermophile var. *dissitum*	Extracellular protease	Adams and Deploey (1978)
Humicola grisea var. *thermoidea*	Extracellular neutral protease	Ong and Gaucher (1973)
H. insolens	Extracellular protease	Adams and Deploey (1978); Satyanarayana and Johri (1983)
H. lanuginosa	Extracellular alkaline protease	Ong and Gaucher (1973), Shenolikar and Stevenson (1982), Satyanarayana and Johri (1983)
Malbranchea pulchella var. *sulfurea*	Extracellular alkaline protease	Ong and Gaucher (1973, 1976)
Mucor miehei	Extracellular acid protease	Rickert and Warren (1974), Lasure (1980)
M. pusillus	Extracellular acid protease	Somkuti and Babel (1967, 1968a) Khan *et al* (1979)
Myriococcum albomyces	Extracellular alkaline protease	Craveri *et al* (1974)
Papulaspora thermophila	Extracellular protease	Adams and Deploey (1978)
Penicillium duponti	Extracellular acid protease	Hashimoto *et al* (1972, 1973), Emi *et al* (1976)
Sporotrichum thermophile	Extracellular protease	Adams and Deploey (1978) Satyanarayana and Johri (1983)
S. pulverulentum	Extracellular acid protease	Eriksson and Pettersson (1982)
Torula thermophila	Extracellular alkaline protease	Karavaeva *et al* (1975)
Thermoascus aurantiacus	Extracellular protease	Satyanarayana and Johri (1983), Adams and Deploey (1978)
Talaromyces thermophilous	Extracellular acid protease	Ong and Gaucher (1973)

only in few instances. *Torula thermophila* UzPT-1 synthesizes maximum enzyme at pH 9–11; *M. pulchella* var. *sulfurea* can synthesize a thermostable extracellular serine protease only in the presence of elastin (Ong and Gaucher 1972) or casein (Ong and Gaucher 1976). Recently, Chouhan (1984) has reported protease production from a thermophilic eumycete Y-15 in wheatbran Czapek-dox broth. pH and temperature optima for this enzyme is 7·4–8 and 45°C, respectively (figure 1). Ong and Gaucher (1973) have reported nearly similar pH optima for enzymes from other thermophilic moulds. As opposed to this Somkuti and Babel (1967) reported an optima of pH 5 for *M. pusillus*. Hashimoto *et al* (1973) reported high yields of an acid protease between pH 4–5·5. Khan *et al* (1979) and Paneerselvam *et al* (1978) observed maximum yields of an acid protease of *M. pusillus* and *A. fumigatus* at pH 4 and 9·5, respectively. Craveri *et al* (1974) found maximum production at pH 7 while Shenolikar and Stevenson (1982) reported that *H. lanuginosa* produced a thiol protease at 7·4 pH.

Somkuti and Babel (1967) and Lasure (1980) have reported maximum yields of protease from *M. pusillus* at 35–37°C. Hashimoto *et al* (1972, 1973) reported maximum accumulation of acid protease of *P. duponti* K-1014 at 50°C, which supports our observations with the thermophilic eumycete Y-15.

Effect of period of incubation has also been examined by several workers. Hashimoto

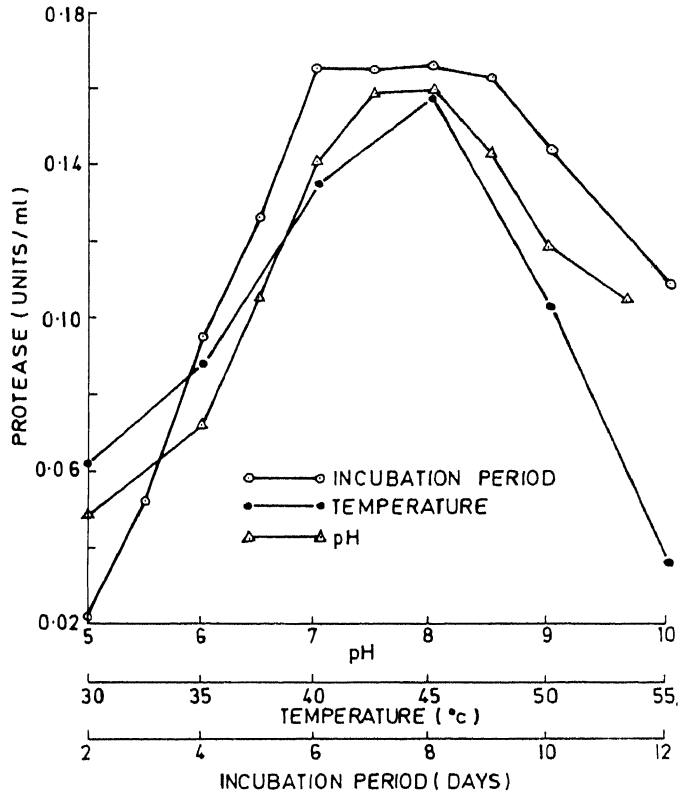


Figure 1. Effect of pH, temperature and incubation period on protease production of thermophilic eumycete Y-15 (Chouhan 1984)

et al (1973) and Paneerselvam *et al* (1978) reported an incubation period of 3 days for protease from *P. duponti* K-1014 and *A. fumigatus*. Craveri *et al* (1974) observed maximum yield of protease by thermophilic eumycete *Myriococcum albomyces* after 74 hr whereas Khan *et al* (1979) reported acid protease production of *M. pusillus* after 92 hr; *H. lanuginosa* produced a thiol proteinase after 36 hr.

4.4 *Purification and characterization*

The separation and purification of proteases of thermophiles has been achieved using conventional protein purification techniques which included ion-exchange chromatography with anion (DEAE cellulose and DEAE sephadex) or cation (cellulose phosphate, CM cellulose etc.) exchangers (Somkuti and Babel 1968a; Chapuis and Zuber 1970; Ong and Gaucher 1973; Gaucher and Stevenson 1976; Emi *et al* 1976; Khan *et al* 1979, 1983; Tapia *et al* 1981), and gel permeation chromatography where the column materials used were usually sephadex and biogel (Karavaeva *et al* 1975, 1976; Emi *et al* 1976; Gaucher and Stevenson 1976; Tapia *et al* 1981; Eriksson and Pettersson 1982). Affinity chromatography using sepharose (4-phenyl-butylamine-czb-D-phenylalanine-TETA), sepharose 4-B, CM sepharose and organomercurial columns were also used

Table 5. Characterization of extracellular protease(s) from thermophilic fungi.

Organism/ type	Optimum pH/ temp./period for production	Optimum pH/ temp. for activity	Thermostability/ pH stability	Inhibitor sensitivity	Mol. wt.	Isoelectric point/Km	References
Mucor pusillus Acid protease	OT = 35°C Op = 5 days	OT = 55°C OpH = 5–6	Lost 90% activity after 15 min exposure at 65°C. Most stable at pH 3–6	Inhibited by most metal ions *i.e.* Hg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Cd^{2+}, Ni^{2+} at conc. 10^{-3} M	—	Km = 0·357% for casein	Somkuti and Babel (1968a)
M. miehei Milk clotting enzyme	—	OpH = 4	Stable at pH 3–6 at 38°C for 8 days	—	38000	PI = 4·2 SD coefficient = S^0_{20} W, 3·35 Svedberg units	Ottesen and Rickert (1970)
Talaromyces dupontii Aminopeptidase Ap-I	—	OT = 65°C OpH = 6·9 for hydrolysis of leucine-*p*-nitro-anilide	Stable at 55°C for 8 hr at pH 7·2, activation by 0·0001 M $CoCl_2$ at 55°C and 65°C	EDTA (0·005 M)	Ap-I = 400000 determined by gel filtration on sepharose 6B	Km = 5 mM at pH 7·2, 65°C for leucine *p*-nitroanilide	Chapuis and Zuber (1970)
Penicillium duponti K-1014 Acid protease	OT = 50°C OpH = 4–5	OpH = 3·5 for haemoglobin and 2·5 for casein	Stable at 60°C for 1 hr at pH 3·5–5·5. 65% activity was lost on exposure at 70°C for 1 hr. Stable at 30°C 2·5–6 pH for 20 hr.	Sod. lauryl sulfate, $KMnO_4$ and N-bromo-succinide	41000 as determined by gel filtration	3·89	Hashimoto *et al* (1973)
Torula thermophila Alkaline protease	OT = 45°C Op = 4 days	OT = 70°C OpH = 8	Highest enzyme resistance at pH 6–8	DFP	—	—	Karavaeva *et al* (1975)
Malbranchea pulchella var. *sulfurea*	OT = 45°C OpH = 7·4	OT = 45°C OpH = 8·5	Remarkably stable in the presence of 10 mM	DFP, PMSF	11000–17000 as determined by gel chromatography	6·0	Ong and Gaucher (1976)

Alkaline protease			Ca^{2+} at pH 7·4. 50% activity was lost at 73°C after 110 min exposure. Stable at pH 4·5–10·5 for 2 hr and 6–9·5 for 20 hr at 30°C.		3200–3000 by dodecyl sulfate gel electrophoresis		
P. duponti K-1014 Acid protease	—	OT = 30°C OpH = 2·5 for casein and 3–3·5 for haemoglobin, egg albumin	Stable at 30°C for 20 hr at pH 2·5–6·5 and for 60°C at pH 3·5–5·5. Most stable at 60°C and 75°C at pH 2·5–3·7	diazo *p*-bromoaceto-phenone, diazoacetyl DL-norleucine methyl ester, and diazoacetyl glycine ethyl ester in the presence of Cu^{2+}	4159 as determined by aminoacid analysis	3·81 as determined by isoelectric focussing	Emi *et al* (1976)
Humicola lanuginosa Thiol proteinase	OT = 45°C Op = 36 hr	OpH = 8 OT = 30°C	—	p-ClHg-BzO, Hg^{+}	23700 Polyacrylamide gel electro-phoresis	—	Shenolikar and Stevenson (1982)
M. pusillus Acid protease and milk clotting enzyme	OT = 35°C OpH = 4 Op = 4 days	Fraction A: milk clotting activity, OpH = 3·8 at 50°C, Fraction B: protease OpH 3·5 at 40°C	Fraction A is more thermostable and less stable under high alkaline range	—	—	—	Khan *et al* (1979)
Sporotrichum pulverulentum Acid protease	OT = 30°C Op = 10 days	OpH for Pro-I = 5 and Pro-II = 5·2. OT = 30°C for both protease fractions	—	Ag^{+}, Hg^{2+} and partial inhibition by EDTA and dipyridyl	Pro-I = 28000 Pro-II = 26000 determined by dodecyl sulfate and PAGE	Pro-I = 4·2 Pro-II = 4·2 by isoelectric focussing and PAGE	Eriksson and Pettersson (1982)

OT = Optimum temperature; OpH = Optimum pH; Op = Optimum period; EDTA = ethylene diamine tetra acetic acid; DFP = diisopropyl phospho fluoriodate; PMSF = phenyl methyl sulfonyl fluoride.

(Ong and Gaucher 1976; Gaucher and Stevenson 1976; Tapia *et al* 1981; Cowan and Daniel 1982; Shenolikar and Stevenson 1982). Further purification utilized isoelectric focussing and crystallization of purified enzymes from ammonium sulphate and acetone (Sawada 1966; Fukumoto *et al* 1967; Kundu and Manna 1975; Emi *et al* 1976; Gaucher and Stevenson 1976).

Kinetics of extracellular proteases especially that pertaining to molecular weight, isoelectric point, Km, substrate specificity and inhibitor sensitivity is summarized in table 5.

pH exerts a profound influence on enzyme activity, and its optima depends upon the nature of proteases (acid, alkaline and neutral). Somkuti and Babel (1968a) reported temperature optima at 55°C and enzyme stability between 3–6 pH for acid protease of *M. pusillus*. Craveri *et al* (1974) characterized the thermostable alkaline protease from thermophilic eumycete *Myriococcum albomyces* and reported an optimum enzyme activity at 55°C and 9 pH. Zakirov *et al* (1975) reported pH optima in alkaline range and temperature optima at 70°C for *T. thermophila*. Satyanarayana and Johri (1983) found pH optima at 6 (figure 2) for the five thermophiles, *viz*, *Achaetomium macrosporum*, *Chaetomium thermophile*, *Sporotrichum thermophile*, *Humicola lanuginosa* and *Torula thermophila*, and temperature optima at 45°C for *A. macrosporum* and *C. thermophile*, 50°C for *S. thermophile* and between 45–50°C in case of *H. lanuginosa* and *T. thermophila* (figure 3). Recently, Chouhan (1984) reported optima for protease of thermophilic eumycete Y-15 at 8–9 pH and 50°C temperature.

Extensive study of proteases of thermophiles has shown that they possess greater thermostability as compared to mesophilic fungal proteases (Somkuti and Babel 1968a;

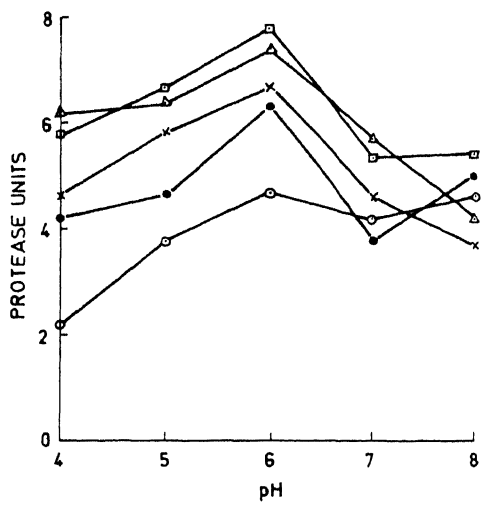


Figure 2. Effect of pH on protease activity of thermophilic fungal strains of decomposing paddy straw. The enzyme activity has been expressed in terms of the liberation of tyrosine.: o—o *Achaetomium macrosporum*, •—• *Chaetomium thermophile*, ×—× *Sporotrichum thermophile*, □ — □ *Humicola lanuginosa*, and, Δ—Δ *Torula thermophila*. (after Satyanarayana and Johri 1983).

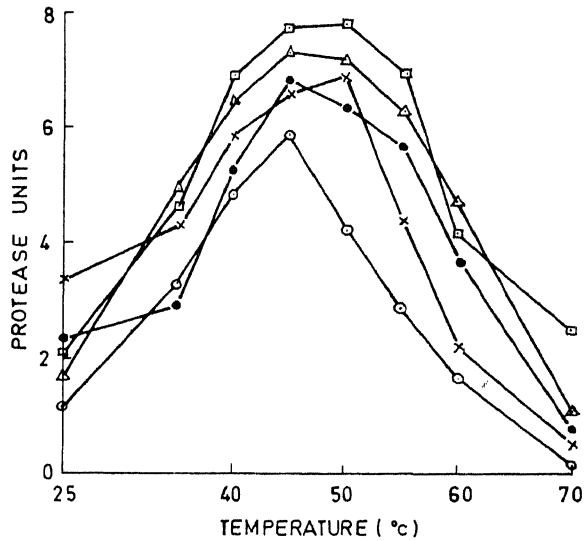


Figure 3. Effect of temperature on protease activity of thermophilic fungal strains of decomposing paddy straw. Temperature optima for enzyme activity was determined by exposing the substrate enzyme assay mixture in a temperature controlled water bath (25–70°C). Other details same as in figure 2. (after Satyanarayana and Johri 1983).

Hashimoto *et al* 1973; Ong and Gaucher 1973; Craveri *et al* 1974; Zakirov *et al* 1975; Satyanarayana and Johri 1983).

Ong and Gaucher (1976) reported that thermomycolase of *M. pulchella* var. *sulfurea* showed more thermostability in the presence of Ca^{2+} ions. Extracellular protease of *H. lanuginosa* and thermophilic eumycete Y-15 is highly thermostable and retained 30–55% activity even after exposure to 70°C (Satyanarayana and Johri 1983; Chouhan 1984). Thermostable extracellular proteases from other thermophiles are also known (Craveri *et al* 1974; Emi *et al* 1976; Karavaeva and Mukhiddinova 1976).

5. Lipases

The extracellular lipases of microbial origin are extremely important since they hydrolyze fats and oils giving rise to free fatty acids and partial glycerides that are essential for metabolic processes. Apart from this, lipases have wide ranging applications in the field of nutrition, food technology, clinical medicine and analytical and preparative chemistry.

5.1 *Classification*

Lipolytic enzymes may be defined as the long-chain fatty acid ester hydrolases. All lipolytic enzymes are hydrolases and as such belong to the class 3 according to the classification recommended by the Enzyme Commission of the International Union of Biochemistry (Florkin and Stotz 1965). Apart from glycosidases, which split the carbohydrate residues from glycolipids, all lipolytic enzymes are ester hydrolases (EC

3.1), with the single exception of ceramidase (EC 3.5). Since lipolytic enzymes hydrolyze esters of fatty acids, they are, therefore, carboxyl ester hydrolases (EC 3.1.1). The phospholipases 3 and 4, are phosphoric diester hydrolases (EC 3.1.4); phosphatidate phosphohydrolase is a phosphoric monoester hydrolase (EC 3.1.3).

5.2 *Application*

The practical utility of these enzymes in food and other related industries has attracted closer attention under uncontrolled conditions, lipases can hydrolyze lipids to produce undesirable rancid flavour in milk and fat containing products. On the other hand, the flavour of cheese is in part due to short chain fatty acids hydrolyzed by the action of microbial and milk lipases. These enzymes also add a characteristic flavour to milk chocolate candy and butter milk pancakes. Other applications of lipases include such diverse processes as degradation of sewage. An acid lipase has been applied in bread dough for the uniform production of monoglycerides that improve its resistance to staling. Such diverse applications indicate commercial significance of controlled and selective lipolysis and suggest additional promising developments.

Glycerol ester hydrolases (EC 3.1.1.3) of moulds in the narrowest sense are the long-chain triglyceride acylhydrolases and several reports of their occurrence exist in literature (Yamada *et al* 1962; Somkuti and Babel 1968b; Somkuti *et al* 1969; Arima *et al* 1972; Hankin and Anagnostakis 1975; Adams and Deploey 1978; Satyanarayana and Johri 1981). However, commercial applications of lipases of microbial origin have aroused renewed interest only in the last decade or so, especially to supplement calf rennet (Fukumoto *et al* 1963, 1964; Lawrence *et al* 1967; Somkuti and Babel 1968b).

Lipolytic fungi have also been implicated in the spoilage of oilseeds under storage (Eggins and Coursey 1964; Apinis and Eggins 1966; Oso 1974a; Kuku and Adeniji 1976; Oso 1979; Ogundero 1981a,b), they can cause a decrease in oil content accompanied by a rapid increase in FFA content which causes deterioration of seeds. Though thermophiles have been reported from a number of oil-rich substrates including grains, crushing sites and oilcakes, but detailed investigations have been carried out only for *Humicola lanuginosa* and *Mucor pusillus* (Somkuti and Babel 1968b; Somkuti *et al* 1969; Arima *et al* 1972). Thermostable lipases of *H. lanuginosa* and others are also a useful complementary tool in the determination of triacetyl glycerol structure since they differ in sites of attack from the better known lipases of animal origin (Alford and Smith 1965). Some important applications of lipases have been summarised in table 3.

5.3 *Production*

Several thermophiles that have been reported to produce lipase include *M. pusillus*, *H. lanuginosa*, *Talaromyces thermophilus*, *H. grisea* var. *thermoidea*, etc. *H. lanuginosa* S-38 produces a remarkable extracellular lipase at 45°C in a medium containing olive oil which has optimum lipolytic activity on polyvinyl alcohol emulsified oil at pH 8 and a temperature of 60°C (Arima *et al* 1972). Lipases of *M. pusillus* and *T. thermophilus* possess peak activity at 45°C while those of *Thermoascus crustaceus* and *H. grisea* var. *thermoidea* are active at 50°C; the pH optima for *H. grisea* var. *thermoidea* and *T. thermophilus* is 5·5 and for *M. pusillus* and *T. crustaceus* at 6 (Ogundero 1980). The optimum temperature for synthesis and activity of *Talaromyces emersonii* lipase has been reported to be 40–45°C (Oso 1978).

Zakirov *et al* (1975) reported lipase activity in a thermotolerant fungus *Rhizopus microsporus* UzLT-1, with optimal temperature 40°C and pH 4·5 for cultivation. Among various metal ions, zinc activated the synthesis of lipase (Sultanova *et al* 1978).

In their ecological study of composting leaves, Hankin *et al* (1976) reported on lipase producing microbes from composting leaves whereas Satyanarayana and Johri (1981) carried out a similar investigation of the thermophilic fungal strains from paddy straw compost. They found that eight thermophiles *viz*, *Absidia corymbifera*, *Acremonium alabamensis*, *Aspergillus fumigatus*, *Chaetomium thermophile*, *Humicola lanuginosa*, *Mucor pusillus*, *Thielavia minor* and *Sporotrichum thermophile*, were able to attack butter and coconut oil which contains saturated fatty acids of 12 carbon atoms, hydroxy fatty acids and fatty acids with conjugated double bonds. This supports the role of lipase producing microbes in the decomposition process besides the fact that thermophiles are in general, more active than the mesophilic mycoflora.

Oso (1974a) recovered seven isolates of thermophilic fungi from stacks of oil palm kernels in Nigeria which comprised of *H. lanuginosa*, *M. pusillus*, *C. thermophile* var. *coprophile*, *C. thermophile* var. *dissitum*, *Thermoascus aurantiacus*, *T. crustaceus* and *Talaromyces emersonii*. Later studies (Oso 1974b) by this worker showed that six isolates could utilize a number of fatty acids as source of carbon. The author concluded that these fungi were capable of taking part in biodeterioration of oil palm kernels produced both during processing and in storage. *T. emersonii* showed induced lipase synthesis when various carbohydrates were used as carbon source both in the presence and absence of yeast extract (Oso 1978).

Studies carried out at our laboratory indicate that the thermophiles isolated from oilseeds (soybean, cotton etc.) stored at room temperature, possess lipolytic activity when tested qualitatively. This shows their possible involvement in the deterioration of oil seeds under storage.

5.4 *Substrate specificity*

Lipases are not inducible in *H. grisea* var. *thermoidea*, *M. pusillus*, *T. thermophilus*, *T. crustaceus* which can synthesize sufficient amounts of enzyme in a medium devoid of lipids. Their addition, however, enhances their production. In general, they have a higher lipolytic activity on synthetic than natural glycerides (Ogundero 1980), but lipase synthesized by *M. pusillus* can hydrolyze both natural (butter fat, vegetable lipids) as well as synthetic triglycerides (Somkuti and Babel 1968b). The lipolytic activity towards synthetic glycerides of low molecular weight has suggested that low fatty acids are present among the hydrolytic breakdown products of butterfat and that, in turn, may be important in the development of hydrolytic rancidity in cheese prepared by *M. pusillus* protease. Additional studies are, therefore, necessary to determine whether mould lipase can be separated from protease or lipase synthesis can be suppressed by manipulating cultivation conditions of *M. pusillus*. Somkuti (1974) investigated the possible removal of lipase activity in concentrated *Mucor* rennet preparations. Gel filtration of enzyme mixtures on sephadex G-75 and sephadex G-100 resulted in a nearly complete resolution of lipase and protease. This might help in preparation of lipase-free fungal rennet.

The definition (Lawrence 1967) of lipase as a 'glycerol ester hydrolase' (EC 3.1.1.3) is applicable also to enzymes isolated from animal systems in which the so-called simple esterases can be distinguished on the basis of their ability to hydrolyze substrates such

as methyl butyrate; triglycerides are hydrolyzed very sluggishly or not at all. Such a definition in regard to microbial lipases, however, needs some explanation, for there is evidence that certain microbial lipases attack both triglycerides and fatty acid esters (Fukumoto *et al* 1964; Iwai *et al* 1964; Lawrence *et al* 1967). Because of this anomaly esterase activity has also been studied in *M. pusillus* lipase. Esterase activity in this, is dependent on the chain length of fatty acid moiety of substrate and is maximal against methyl dodecanoate. This finding contrasts from the data reported earlier on glycerol ester hydrolase activity which was maximum when fatty acid moiety in triglyceride has 8 carbon atoms (trioctanoin); with the exception of methyl butyrate, the enzyme from *M. pusillus* hydrolyzes all the tested fatty acid methyl esters. Thus it appears that a minimum chain length of 6 carbons is required by this enzyme to hydrolyze simple esters. A similar requirement was reported for lipase of *A. niger* which, with the exception of methyl butyrate, also hydrolyzed simple esters in addition to triglycerides (Iwai *et al* 1964). On the other hand, lipase of *Rhizopus delemar* can hydrolyze methyl butyrate as well (Fukumoto *et al* 1964).

5.5 *Thermal inactivation*

Lipolytic activity in the reaction mixture depends upon a series of factors which in single or combination can influence this system. The effect of surface active agents on lipase reaction of *H. lanuginosa* has been shown to be complex (Liu *et al* 1973a) and depends upon the chemical nature of the agents used. The physical condition of the substrate necessary for lipolytic activity also varies with the source of lipase (Liu *et al* 1973b). Sulphydryl reducing agents like dithiothreitol cannot alter the enzyme activity but renders it more susceptible to heat inactivation. Addition of urea accelerates thermal inactivation of the enzyme. The inhibition of its action on olive oil by various agents is not due to inactivation of the enzyme directly but due to displacement of the substrate from the oil/water interface which blocks the enzyme from reaching the substrate (Liu *et al* 1973a). Reducing compounds (cysteine, 2-mercaptoethanol), chelating agents (EDTA, *o*-phenanthroline) and thiol group of poisons (*p* CMB, monoiodoacetate) show no detectable effect on glycerol ester hydrolase or esterase activity of *M. pusillus*, indicating that lipase is not a metallo-enzyme and does not require either a free-SH group(s) or an intact S-S bridge(s) for its activity.

The crude lipase of *M. pusillus*, when heated at 55 and 58°C for 10, 24, 45 min, showed that the enzyme lost 10, 27 and 50% of its activity, respectively (Somkuti and Babel 1968b). While the enzyme of *H. lanuginosa* S-38 is highly thermostable, as it retained 100% activity at 60°C for 2 hr, however, at 70°C for 20 min, only 35% activity remained.

Craveri *et al* (1974) isolated a thermostable and alkalistable lipolytic enzyme from a thermophilic eumycete, *Myriococcum albomyces*. This enzyme was stable between pH 4, 5 and 8, after 1 hr at 35°C it retained 100% activity; after 30 min at 55°C the enzyme activity was reduced to about 80%.

6. Newer approaches

Immobilized systems have found wide ranging applications in the modern era, since they by-pass many of the short comings which frequently make soluble enzymes commercially impractical. Their superiority comes from cheaper cost due to reuse,

improved stability and from the possibility to influence the efficiency of the catalyst by modifying its microenvironment. They prevent allergenic responses or other undesired interactions between enzyme and specific components of the reaction mixture including contamination of the final product by the enzyme. Use of immobilized system provides better control through proper reactor design; the effective concentrations of substrate or of products as well as the sequential action of catalysts in multi-step processes is facilitated.

The applications of immobilized enzymes in processing and analyses of milk products appears promising because of the characteristics of milk systems. Most milk products are fluids during processing stages, and continuous processing systems i.e., cheese manufacture, are being developed which could utilize immobilized enzymes. In addition, modification of milk constituents, such as fat with immobilized enzymes would seem feasible and advantageous.

Immobilized proteases might be valuable in continuous coagulation of milk for cheese manufacture. Since immobilized enzymes would not remain in the product, it may be possible to substitute a less expensive and less desirable, but more readily available enzyme which normally cannot be used commercially as a milk clotting enzyme.

The potential of immobilized microbial rennets has not been much exploited. However, once continuous clotting processes become operative for actual cheese production, the better stability of microbial rennets compared to animal enzymes will make them more attractive for use in immobilized form. Substitution of calf rennet by microbial rennets is already an acknowledged facet because of its higher cost, less availability and also from a religious point of view. Moreover, a term now being used for cheese produced through microbial rennets in vogue is "vegetarian cheese".

Acknowledgements

Some of the unpublished work reported in this review has been carried out with support from ICAR and CSIR which is gratefully acknowledged.

References

Abbott B J 1976 Preparation of pharmaceutical compounds by immobilized enzymes and cells; *Adv. Appl. Microbiol.* **20** 203–257

Adams P R and Deploey J J 1976 Amylase production by *Mucor miehei* and *Mucor pusillus*; *Mycologia* **68** 934–938

Adams P R and Deploey J J 1978 Enzymes produced by thermophilic fungi; *Mycologia* **70** 906–910

Alford J A and Smith J L 1965 *J. Am. Oil Chem. Soc.* **42** 1038–1040

Apinis A E and Eggins H O W 1966 *Thermomyces ibadanensis* sp. nov from oil palm kernel stacks in Nigeria; *Trans. Br. Mycol. Soc.* **49** 629–632

Arima K, Iwasaki S and Tamura G 1967 Milk clotting enzymes from micro-organisms 1. Screening test and the identification of the potent fungus; *Agric. Biol. Chem.* **31** 540–545

Arima K, Iwaski J, Yu S and Tamura G 1968 Milk clotting enzymes from micro-organisms V. Purification and crystallization of Mucor rennin from *Mucor pusillus* var. Lindt; *Appl. Microbiol.* **16** 1727–1733

Arima K, Liu W H and Beppu T 1972 Studies on the lipase production of thermophilic fungus *Humicola lanuginosa*; *Agric. Biol. Chem.* **36** 893–895

Arnold R G, Shahani K M and Dwivedi B K 1975 Application of lipolytic enzymes to flavor development in dairy products; *J. Dairy Sci.* **58** 1127–1143

Aunstrup K 1971 Industrial production of proteolytic enzymes; in *Industrial aspects of biochemistry* (ed.) B Spencer (Amsterdam, London: North-Holland) **30** 23–43

Aunstrup K 1977 *Annual reports on fermentative processes* (eds) D Perlman and G T Tsao (New York: Academic Press) vol I pp. 181–204

Canevascini G and Meyer H P 1979 β-glucosidase in the cellulolytic fungus *Sporotrichum thermophile* Apinis; *Exp. Mycol.* **3** 203–214

Chakrabarty S L and Sen S 1982 Characteristics of the cellulase produced by *Myceliophthora thermophila; Can. J. Microbiol.* **28** 271–277

Chapman E S, Evans E, Jacobelli M C and Logan A A 1975 The cellulolytic and amylolytic activity of *Papulaspora thermophila*; *Mycologia* **67** 608–615

Chapman E S, Evans E, Jacobelli M C and Logan A A 1975 The cellulolytic and amylolytic activity of *Papulaspora thermophila*; *Mycologia* **68** 678–681

Chapuis R and Zuber H 1970 Thermophilic aminopeptidases: AP-I from *Talaromyces dupontii*; in *Methods in enzymology* **19** 552–555

Charles R L, Gertzman D P and Melachouris N 1970 U.S. Patent 3549390

Chouhan S 1984 *Extracellular proteases of thermophilic fungi.* Ph.D. Thesis; Bhopal University, Bhopal (unpublished)

Cowan D A and Daniel R M 1982 Purification and some properties of an extracellular protease (caldolysin) from an extreme thermophile; *Biochim. Biophys. Acta.* **705** 293–305

Coughlan M P and Hale A Mc 1981 The cellulolytic system of *Talaromyces emersonii* identification of the various components produced during growth of cellulosic media; *Biochim. Biophys. Acta.* **662** 145–151

Coutts A D and Smith R E 1976 Factors influencing the production of cellulase by *Sporotrichum thermophile; Appl. Environ. Microbiol.* **31** 819–825

Craveri R, Manachini P L and Aragozzini F 1974 Thermostable and alkaline lipolytic and proteolytic enzymes produced by a thermophilic mold; *Mycopathol. Mycol. Appl.* **54** 193–204

Davies R 1963 Microbiol extracellular enzymes: their use and some factors affecting their formation; in *Biochemistry of industrial micro-organisms* (eds) C Rainbow and A H Rose (Inc. London: Academic Press) pp. 68–150

Dion W M 1950 The proteolytic enzymes of micro-organisms I. Survey of fungi and actinomycetes for protease production in submerged culture; *Can. J. Res.* **C28** 577–585

Eggins H O W and Coursey D G 1964 Thermophilic fungi associated with Nigerian oil palm produce; *Nature (London)* **203** 1083–1084

Emi S, Meyers D V and Iacobucci G A 1976 Purification and properties of the thermostable acid protease of *Penicillium duponti*; *Biochem. J.* **15** 842–848

Endo T 1962 Studies on protease produced by thermophilic bacteria; *J. Ferment. Technol.* **40** 346–353

Eriksen J and Goksoyr 1976 The effect of temperature on growth and cellulase (β-1-4-endo-glucanase) production in the compost fungus *Chaetomium thermophile* var. *dissitum.*; *Arch. Mikrobiol.* **110** 233–238

Eriksson K E and Pettersson B 1982 Purification and partial characterization of two acidic protease from the White rot fungus *Sporotrichum pulverulentum*; *Eur. J. Biochem.* **124** 635–642

Fergus C L 1964 Thermophilic and thermotolerant molds and actinomycetes of mushroom compost during peak heating; *Mycologia* **56** 267–284

Fergus C L 1969 The cellulolytic activity of thermophilic fungi and actinomycetes; *Mycologia* **61** 120–129

Fergus C L and Sinden J W 1969 A new thermophilic fungus from mushroom compost *Thielavia thermophila* sp. nov; *Can. J. Bot.* **47** 1635–1637

Flannigan B and Sellars P N 1972 Activities of thermophilous fungi from barley kernels against arabinoxylan and carboxymethyl cellulose; *Trans. Br. Mycol. Soc.* **58** 338–341

Flannigan B and Sellars P N 1978 Production of xylolytic enzymes by *Aspergillus fumigatus; Trans. Br. Mycol. Soc.* **71** 353–358

Florkin M and Stotz E H (ed.) 1965 *Comprehensive biochemistry* (Amsterdam: Elsevier) vol 12

Fogarty W M and Kally C T 1978 in *Topics in enzyme and fermentation biotechnology* (ed.) Allen Wiseman (New York: Halsted Press) pp. 45–86

Fukumoto J, Iwai M and Tsujisaka Y 1963 Studies on lipase, I. Purification and crystallization of a lipase secreted by *Aspergillus niger*; *J. Gen. Appl. Microbiol. (Tokyo)* **9** 353–361

Fukumoto J, Iwai M and Tsujisaka Y 1964 Studies on lipase IV. Purification and properties of lipase secreted by *Rhizopus delemar; J. Gen. Appl. Microbiol. (Tokyo)* **10** 257–265

Fukumoto J, Tsuru D and Yamamoto T 1967 Studies on mold proteases. I. Purification, crystallization and some enzymic properties of acid protease of *Rhizopus chinensis*; *Agric. Biol. Chem.* **31** 710–717

Gaucher G M and Stevenson K 1976 Thermomycolin; in *Methods in enzymology* (ed.) L Lorand (New York: Academic Press) Vol 65 415–433

Gutcho J 1974 *Microbiol enzyme production* (Park Ridge, New Jersey: Noyes Data Corp.) pp. 272

Hankin L and Anagnostakis S L 1975 The use of solid media for detection of enzyme production by fungi; *Mycologia* **67** 597–607

Hankin L, Pioncelot R P and Anagnostakis S L 1976 Micro-organisms from composting leaves: ability to produce extracellular degradative enzymes; *Microbial Ecol.* **2** 296–308

Hashimoto H, Iwaasa T and Yokotsuka T 1972 Thermostable acid protease produced by *Penicillium duponti* K-1014, a true thermophilic fungus newly isolated from compost; *Appl. Microbiol.* **24** 986–992

Hashimoto H, Iwaasa T and Yokotsuka T 1973 Some properties of acid protease from the thermophilic fungus, *Penicillium duponti* K-1014; *Appl. Microbiol.* **25** 578–582

Hayashida S 1980 Production and purification of thermostable cellulases from *Humicola insolens* YH-8; *Agric. Biol. Chem.* **44** 1721–1728

Hayashida S and Hungo 1968 Abstr. Papers. *Ann. Meet. Agric. Chem. Soc. Jpn.* pp. 204

Hayashida S and Yoshioka H 1980 Production and purification of thermostable cellulases from *Humicola insolens* YH-8; *Agric. Biol. Chem.* **44** 1721–1728

Iwai M, Tsujisaka Y and Fukumoto J 1964 Studies on lipase. II. Hydrolytic and esterifying actions of crystalline lipase of *Aspergillus niger; J. Gen. Appl. Microbiol. (Tokyo)* **10** 13–22

Iwasaki S, Tamura G and Arima K 1967 Milk clotting enzyme from micro-organisms. II. The enzyme production and properties of crude enzyme; *Agric. Biol. Chem. (Tokyo)* **31** 546–551

Iwasaki T and Kosikowski F V 1973 Increasing flavor in cheese with commercial microbial enzyme preparations; *J. Dairy Sci.* **56** 623 (Abstr)

Jain M K, Kapoor K K and Mishra M M 1979 Cellulase activity, degradation of cellulose and lignin, and humus formation by thermophilic fungi; *Trans. Br. Mycol. Soc.* **73** 85–89

Johnson A G and Martin S M 1964 Protease production by *Aspergillus fumigatus; Agric. Biol. Chem. (Tokyo)* **28** 734–739

Karavaeva N N and Mukhiddinova N G 1976 Multiplicity of molecular forms and thermal stability of protease of the fungus *Torula thermophila* strain UzPT-1; *Biokhimiya* **47** 1625–1630

Karavaeva N N, Zakirov M N and Mukhiddinova N G 1975 Partial purification and some properties of protease from *Torula thermophila; Biokhimiya* **40** 909–914

Keay L 1971 Microbial proteases; *Process Biochem.* **6** 17–21

Khan M R, Blain J A and Patterson J D E 1979 Extracellular proteases of *Mucor pusillus; Appl. Environ. Microbiol.* **37** 719–724

Khan M R, Blain J A and Patterson J D E 1983 Partial purification of *Mucor pusillus* intracellular proteases; *Appl. Environ. Microbiol.* **45** 94–96

Kosikowski F V and Iwasaki T 1975 Changes in cheddar cheese by commercial enzyme preparations; *J. Dairy Sci.* **58** 963–970

Kuku F O and Adeniji M O 1976 The effects of moulds on the quality of Nigerian palm kernels; *Int. Biodeterior. Bull.* **12** 37–41

Kundu A K and Manna S 1975 Purification and characterization of extracellular proteinases of *Aspergillus oryzae; Appl. Microbiol.* **30** 507–513

Lasure L L 1980 Regulation of extracellular acid protease in *Mucor miehei; Mycologia* **72** 483–493

Lawrence R C 1967 Microbiol lipases and related esterases. I. Detection, distribution and production of microbiol lipases; *Dairy Sci. Abstr.* **29** 8–15

Lawrence R C, Fryer T F and Reiter B 1967 The production and characterization of lipases from a *Micrococcus* and a *Pseudomonas; J. Gen. Microbiol.* **48** 401–418

Liu W H, Beppu T and Arima K 1972 Cultural conditions and some properties of the lipase of *Humicola lanuginosa* S-38; *Agric. Biol. Chem.* **36** 1919–1924

Liu W H, Beppu T and Arima K 1973a Effect of various inhibitors on lipase action of thermophilic fungus *Humicola lanuginosa* S-38; *Agric. Biol. Chem.* **37** 2487–2492

Liu W H, Beppu T and Arima K 1973b Physical and chemical properties of the lipase of thermophilic fungus *Humicola lanuginosa* S-38; *Agric. Biol. Chem.* **37** 2493–2499

Luis A J and Beaker R R 1973 The B-glucosidase system of thermophilic fungus *Chaetomium thermophile* var. *coprophile; Biochem. Biophys. Acta* **329** 5–19

Maheshwari R, Balasubramanyam P V and Palanivelu P 1983 Distinctive behaviour of invertase in a thermophilic fungus *Thermomyces lanuginosus; Arch. Mikrobiol.* **134** 255–260

Margaritis A, Marchant R and Yaguchi M 1983 Xylanase, C-M cellulase and avicelase production by the thermophilic fungus *Sporotrichum thermophile; Biotech. Lett.* **5** 265–270

Morihara K 1974 Comparative specificity of microbiol proteinases; *Adv. Enzymol.* **41** 179–243

Mukherjee M and Chaudhari K L 1977 Studies on protease production in *Penicillium javanicum* var. *beyma*; *Curr. Sci.* **46** 151–153

Nakanishi T and Itoh M 1974 Enzymatic studies on cheese ripening X. Changes in volatile free fatty acids, carbonyls, and sulfur compounds during ripening of cheese made with added protease produced by *Aspergillus oryzae* strain B; *Jpn J. Dairy Sci.* **23** A 181

Neurath H 1957 The multiple specificity of proteolytic enzymes; *Ann. N. Y. Acad. Sci.* **68** 11–24

Nishio N, Kurisu H and Nagai S 1981 Thermophilic cellulase production by *Talaromyces* sp. in solid state cultivation; *J. Ferment. Technol.* **59** 407–410

Ogundero V W 1979 Amylase and cellulase activities of thermophilic fungi causing deterioration of tobacco products in Nigeria; *Mycopathology* **69** 131–136

Ogundero V W 1980 Lipase activity of thermophilic fungi from mouldy groundnuts in Nigeria; *Mycologia* **72** 118–126

Ogundero V W 1981a Isolation of thermophilic and thermotolerant fungi from stored groundnuts in Nigeria and determination of their lipolytic activity; *Int. Biodeterior. Bull.* **17** 51–56

Ogundero V W 1981b Thermophilic fungi from Nigerian palm produce; *Mycologia* **73** 198–202

Ohta Y 1967 Thermostable protease from thermophilic bacteria; *J. Biol. Chem.* **242** 509-515

Oka T, Ishino K, Tsuzuki K, Morihara K and Arima K 1973 Specificity of a rennin like enzyme from *Mucor pusillus*; *Agric. Biol. Chem.* **37** 1177–1184

Oleniacz W S and Pisano M A 1968 Proteinase production by a species of *Cephalosporium*; *Appl. Microbiol.* **16** 90–96

Olutiola P O 1982 Characterization of cellulase from *Humicola lanuginosa*; *Experientia* **38** 1332–1333

Ong P S and Gaucher G M 1973 Protease production by thermophilic fungi; *Can. J. Microbiol.* **19** 129–133

Ong P S and Gaucher G M 1972 Production, purification and partial characterization of an extracellular protease from a thermophilic fungus; *Proc 4th Int. Ferment. Symp.* Kyoto, Japan 271–278

Ong P S and Gaucher G M 1976 Production, purification and characterization of thermomycolase, the extracellular serine protease of the thermophilic fungus *Malbranchea pulchella* var. *sulfurea*; *Can. J. Microbiol.* **22** 165–176

Oso B A 1974a Thermophilic fungi from stacks of oil palm kernels in Nigeria; *Z. Allg. Mikrobiol.* **14** 593–601

Oso B A 1974b Utilization of lipids as sole carbon source by thermophilic fungi; *Z. Allg. Mikrobiol.* **14** 713–717

Oso B A 1978 The lipase activity of *Talaromyces emersonii*; *Can. J. Bot.* **56** 1840–1843

Oso B A 1979 Mycelial growth and amylase production by *Talaromyces emersonii*; *Mycologia* **71** 520–529

Ottesen M and Rickert W 1970 The acid protease of *Mucor miehei*; in *Methods in enzymology* (ed.) G E Perlman (New York: Academic Press) Vol 19 pp. 459–460

Paneerselvam M and Dhar S C 1980 Chromatography, purification and homogeneity of extracellular acid proteinase of *Aspergillus fumigatus*; *Ital. J. Biochem.* **29** 102–112

Paneerselvam M, Dhar S C and Santappa M 1978 Factors influencing the production of extracellular proteinases by *Aspergillus fumigatus*; *Leather Sci.* **25** 499–506

Patil M and Shastri N V 1981 Extracellular production of protease by *Alternaria alternata* (Fr) Keissler; *J. Ferment. Technol.* **59** 403–406

Prasad A R S and Maheshwari R 1979 Temperature response of trehalase from a mesophilic (*Neurospora crassa*) and a thermophilic (*Thermomyces lanuginosus*) fungus; *Arch. Microbiol.* **122** 275–280

Premabai M and Narasimha Rao P L 1963 Thermophilic micro-organisms I. Isolation and morphological characteristics of *Malbranchea pulchella*; *Indian J. Exp. Biol.* **1** 50–53

Prins J and Nidson T K 1970 *Process Biochem.* **5** 34–35

Ramabadran R 1970 Production of amylase and protease by thermophilic fungi; *Annamalai Univ. Agric. Res. Ann.* **2** 45–47

Ramaley R F 1972 *Fed. Proc.* **31** A 478

Rao U B and Maheshwari R 1979 A thermostable glucoamylase from the thermophilic fungus *Thermomyces lanuginosus*; *Curr. Sci.* **48** 113–115

Rao U B and Shastri N V S 1981 Purification and characterization of a thermostable glucoamylase from the thermophilic fungus *Thermomyces lanuginosus*; *Biochem. J.* **193** 379–387

Reed G 1975 *Enzymes in food processing.* 2nd edn (New York: Academic Press) pp. 573

Richardson G H, Nelson J H, Lubnow R E and Schwarberg R L 1967 Rennin-like enzyme from *Mucor pusillus* for cheese manufacture; *J. Dairy Sci.* **50** 1066–1072

Rickert W S and Mc Bride-Warren P A 1974 Structural and functional determinants of *Mucor miehei* protease III. Isolation and composition of the carbohydrate moiety; *Biochim. Biophys. Acta.* **336** 437–444

Rosenberg S L 1978 Cellulose and lignocellulose degradation by thermophilic and thermotolerant fungi; *Mycologia* **70** 1–13

Sardinas J L 1968 Rennin enzymes of *Endothia parasitica*; *Appl. Microbiol.* **16** 248–255

Sardinas J L 1972 Microbiol rennets; *Adv. Appl. Microbiol.* **15** 39–81

Satyanarayana T 1978 *Thermophilic microorganisms and their role in composting process* Ph.D. Thesis University of Sagar, Sagar

Satyanarayana T and Johri B N 1981 Lipolytic activity of thermophilic fungi of paddy straw compost; *Curr. Sci.* **50** 680–682

Satyanarayana T and Johri B N 1983 Extracellular protease of thermophilic fungi isolated from decomposing paddy straw; *Trop. Plant. Sci. Res.* **1** 137–140

Sawada J 1966 *Agric. Biol. Chem.* **30** 393

Sen S K, Abraham T K and Chakrabarty S L 1981 Cellulolytic activity of *Myceliophthora thermophila* D-14; *Curr. Sci.* **50** 598–600

Shenolikar S and Stevenson K J 1982 Purification of thiol proteinase from the thermophilic fungus *Humicola lanuginosa; Biochem. J.* **205** 147–152

Singh P 1982 *Thermophilous microfungi of soil and air and their enzymatic activity* Ph.D. Thesis, Bhopal University, Bhopal

Singh K and Martin S M 1960 Purification and properties of a protease from *Penicillium cyaneo-fulvum*; *Can. J. Biochem. Physiol.* **38** 969–980

Somkuti G A 1974 Simple method for removal of lipase from *Mucor* rennet; *J. Dairy Sci.* **57** 591 (*Abstr.*)

Somkuti G A and Babel F J 1967 Conditions influencing the synthesis of acid protease by *Mucor pusillus* Lindt.; *Appl. Microbiol.* **15** 1309–1312

Somkuti G A and Babel F J 1968a Purification and properties of *Mucor pusillus* acid protease; *J. Bacteriol.* **95** 1407–1414

Somkuti G A and Babel F J 1968b Lipase activity of *Mucor pusillus; Appl. Microbiol.* **16** 617–619

Somkuti G A, Babel F J and Somkuti A C 1969 Lipase of *Mucor pusillus*; *Appl. Microbiol.* **17** 606–610

Somkuti G A and Steinberg D H 1980 Thermoacidophilic extracellular α-amylase of *Mucor pusillus*; *Rev. Ind. Microbiol.* **21** 327–337

Sood V K and Kosikowski F V 1979 Accelerated cheddar cheese ripening by added microbial enzymes; *J. Dairy Sci.* **62** 1865–1872

Sternberg M 1972 Bond specificity, active site and milk clotting mechanism of the *Mucor miehei* protease; *Biochem. Biophys. Acta* **285** 383–392

Sternberg M 1976 Microbial rennets; *Adv. Appl. Microbiol.* **20** 135–157

Streamer M, Eriksson K E and Pettersson B 1975 Extracellular enzyme system utilized by the fungus *Sporotrichum pulverulentum* for the breakdown of cellulose; *Eur. J. Biochem.* **59** 607–613

Subrahmanyam A, Mangallam S and Gopal Krishnan K S 1977 Amyloglucosidase production by *Torula thermophila*; *Indian J. Exp. Biol.* **15** 495–496

Sultanova I G, Zubenko T F and Zakirov M Z 1978 The effect of metal ions on the lipolytic activity of *Rhizopus microsporus*; *Mikrobiologiya* **47** 1010–1013

Tapia G, Curotto E, O'Reilly S and Gohzalez G 1981 Isolation and partial characterization of an extracellular protease from *Sporotrichum dimorphosporum; FEBS Letters* **130** 205–206

Taylor P M, Napier E J and Fleming I D 1978 Amyloglucosidase production and purification from thermophilic fungus *Humicola lanuginosa*; in *Carbohydrate Res.* **61** 301–308

Tong C C and Cole Al J 1982 Cellulase production by thermophilic fungus *Thermoascus aurantiacus; Pertanika* **5** 255–262

Underkofler L A 1976 *Industrial microbiology* (eds) B M Miller and W Litsky (New York: McGraw Hill) pp. 128–164

Vandamme E J, Loggle J M and Geeraests H A M 1982 Cellulase activity of a thermophilic *Aspergillus fumigatus* (Fresenius) strain; *J. Chem. Technol. Biotechnol.* **32** 968–974

Voordouw G and Roche R S 1975 The role of bound calcium ions in thermostable proteolytic enzymes I. Studies on thermomycolase, the thermostable protease from the fungus *Malbranchea pulchella* var. *sulfurea; Biochem. J.* **14** 4659–4666

Wang H L and Hesseltine C W 1965 Studies on extracellular proteolytic enzymes of *Rhizopus oligosporus; Can. J. Microbiol.* **11** 727–732

Wiseman A 1975 *Handbook of enzyme biotechnology* (New York: Halsted Press) pp. 275

Yamada K, Ota Y and Machida H 1962 Studies on the production of lipase by micro-organisms II. Quantitative determination of lipase; *J. Agric. Chem. Soc. Jpn.* **36** 860–864

Yoshioka H, Chavanich S, Nilubol N and Hayashida S 1981 Production and characterizatio thermostable xylanase from *Talaromyces byssochlamydoides* YH-50; *Agric. Biol. Chem.* **45** 579–58
Yu J, Tamura G and Arima K 1969 *Biochem. Biophys. Acta* **171** 138–144
Zakirov M Z, Shchrlokova S S and Karavaeva N N 1975 *Torula thermophila* UzPT-1 thermophilic fu producing proteolytic enzymes; *Appl. Biochem. Microbiol.* **21** 608–612

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 197–207.
© Printed in India.

Physiological studies of the Gymnoascaceae with an overview on their ecology

GOURI RANI GHOSH
Department of Microbiology, Maulana Azad Medical College, New Delhi 110002, India

Abstract. The family Gymnoascaceae, is related to the dermatophytes. Anamorphs of certain members lie in *Chrysosporium*, *Microsporum* and *Trichophyton*. The members of the family are world wide in distribution. They live as saprophytes on various Keratinous substrates, dung, droppings and in soil. Often they are isolated from clinical sources of human and animal. Therefore these fungi have been regarded as "potential pathogens and opportunistics". Thus the family is important. Studies on Gymnoascaceae have been mostly confined to morphological and taxonomical details. Investigations on the physiology of its various members are limited, though such studies are called for. The present paper reviews the physiological studies so far conducted in the family dating back to the year 1893. The early investigators confined their studies to nutritional requirements of certain species. Later workers employed nutritional biochemical behaviour of different species and strains as a parameter for clarifying taxonomic confusions. The biochemical behaviour is genetically controlled and may have a bearing on taxonomy. More recently a theoretical study was undertaken to analyse the growth patterns quantitatively. Equations for growth curves of certain species were obtained. The relevance of undertaking such numerical methods is appreciated in view of the facts that these predictions can give clues on certain taxonomical and behavioural patterns.

Keywords. Gymnothecium; aleuriospores; arthro-aleuriospore; racquetmycelia; fuseaux; teleomorph; opportunistic; biochemical behaviour; parameter.

1. Introduction

The family Gymnoascaceae was established in 1872 by Baranetzky, a German Mycologist, based on his new fungus *Gymnoascus reessii*. In 1893, Schroeter published the established concepts of the family which have remained essentially unchanged to date. The Gymnoascaceae is a primitive family characterised by a simple ascocarp designated as "Gymnothecium" by Novak and Galgoczy (1966) which either lacks a peridium or possesses one composed of loosely interwoven hyphae. It is classified under the division-Eumycota; sub-division-Ascomycotina; Class-Plectomycetes; Order-Eurotiales; Family-Gymnoascaceae.

The anamorph (asexual phase), if present usually consists of oidia, aleuriospores, arthro-aleuriospores or conidia of various types similar to those of dermatophytes or Trichophytoneae and includes *Chrysosporium*, *Microsporum* and *Trichophyton*. When grown on similar media both Gymnoascaceae and Trichophytoneae produce similar and comparable structures such as racquet-mycelia, microconidia, fuseaux or macroconidia.

Matruchet and Dassonville (1899, 1900, 1901) first suggested that the dermatophytes have their perfect stage in Gymnoascaceae and should be classified with them. Since then this family has attracted the attention of several investigators. Subsequent workers reported perfect state (teleomorph) formation in *Microsporum gypseum* (Griffion 1960a, b; Stockdale 1961). Discoveries of sexual stages of dermatophytes in

Gymnoascaceae proved beyond doubt that some members of the family were the teleomorphs of certain dermatophytes and others pathogenic fungi (Dawson and Gentles 1961; Rioux *et al* 1966; Ashbrook 1968; Mc Donough and Lewis 1968; Kwon-Chung 1972; Sigler *et al* 1979) Pseudocliestothecia production by strains of *Trichophyton rubrum* has been reported by Young (1968) and Ghosh *et al* (1976).

2. Ecology and biology

Members of the Gymnoascaceae are world wide in distribution, though reported largely from tropical and subtropical regions. These fungi mostly live as saprophytes on a variety of substrates including feather, fur, nail, hoof, horn, bone, dung, droppings, decaying vegetable matter, paper and soil. Very often they have been isolated from clinical sources of human and animal (Matruchot and Dassonville 1901; Castellani 1937; Delamater 1937; Emmons 1954; Kuehn 1957; Kuehn *et al* 1961, 1964; Orr *et al* 1963, 1977; Roy and Chunder 1963; De Vroey 1966, 1970; Ghosh *et al* 1976). Some authors describe them as 'opportunistic' fungi (Emmons 1962; Ajello 1962). According to Kuehn *et al* (1964) consideration of some species of Gymnoascaceae as "opportunistic" fungi is appropriate for at least two reasons; it contains the teleomorphs (ascocarpic stages) of certain known disease agents and the teleomorphs themselves have been isolated from pathological materials from different types of lesions. Therefore, these fungi have been regarded as at least "potential pathogens" (Kuehn *et al* 1964; Roy *et al* 1972).

Current opinion on the mode of life of the dermatophytes is divided. One school of thought believes that certain dermatophytes have adjusted exclusively to a parasitic mode of life in evolution, though attempts to make them live as saprophytes on various culture media and even on soil have been successful *in vitro*. Others believe that it is not wise to reject the hypothesis of a saprophytic mode of life of these organisms though attempts to isolate specific dermatophytes from the environment have been unsuccessful. Perhaps the reason for this may be either that their actual 'biotope' is not known or the exact type of keratin bait for their isolation still remains unknown. (Vanbreuseghem 1973). By using selective keratin baits we have been successful in isolating new members of the Gymnoascaceae from soil (Ghosh 1983).

What has been stated above for the dermatophytes can apply to the Gymnoascaceae as well. The series of discoveries of perfect stages of some keratinophilic fungi and dermatophytes in the family Gymnoascaceae enhanced this view.

According to De Vroey (1966) *Pseudoarachniotus roseus* was widely distributed in Somalia and was isolated from 8·5% of clinical samples.

However, De Vroey could not prove conclusively the pathogenic role of *P. roseus* and considered it as a "weak pathogen". The species *P. roseus* is described more than any other species of the Gymnoascaceae. It is isolated from the greatest variety of substrates and is most widely distributed geographically. Large numbers have been isolated from clinical sources. Isolation reports of *P. flavoluteus*, (a closely related species) from clinical sources are limited. Orr *et al* (1977) for the first time reported this species from such a source. Sur and Ghosh (1980) reported its existence in nature as geophilic-Keratinophilic fungi having affinity for sparrow and chicken feathers.

These geophilic-Keratinophilic group of fungi are to be recognised as "potential pathogens" since they possess the ability to grow on Keratinous baits. Ghosh and

Sarangi (1983) while surveying Keratinophilic fungi inhabiting wild and free living *Passer domesticus* in Orissa (India), observed that these birds act as mechanical carrier of some disease agents without themselves being actually infected. The distribution pattern of Keratinophilic fungi in positive samples (77·6%) from common house sparrow recorded 6·47% incidence of the fungus *P. flavoluteus*.

3. Physiology

Studies on the Gymnoascaceae have been mainly confined to morphological and taxonomical details. These works have been summarised or reviewed by Kuehn (1955a, b), Benjamin (1956), Apinis (1964), De Vroey (1970), von Arx (1971), Fennell (1973) and Orr *et al* (1977).

Though Gymnoascaceae is an important family, studies on the physiology of its various members are limited, whereas a number of physiological studies have been reported on the related groups *viz*, the Trichophytoneae (dermatophytes) and the Aspergillaceae.

Interest in the physiology of Gymnoascaceae dates back to 1893. According to Latham (1909), Berthelot in 1893 claimed that an unidentified species of *Gymnoascus* was capable of fixing atmospheric nitrogen. However, serious investigations on the nutrition of the Gymnoascaceae apparently started with Volkonosky (1934) and continued with Hawker (1936), Schopfer and Blumer (1940), Nyuksha (1953, 1955, 1957), Kuehn and Crosby (1959, 1960a–d), Ghosh (1954, 1960), Kuehn (1961, 1962), Kuehn and Orr (1962). Volkonosky (1934) compared the nutrition of *Ctenomyces serratus* Eidam, a saprophyte, with those of certain dermatophytes, including *Sabouraudites felineus* Ota and Langeron (= *Ectotrichophyton felineum* (Blanchard) Castellani and Chalmers), *Epidermophyton floccosum* (Harz) Langeron and Milochevitch, *Trichophyton crateriforme* Sab. (= *T. tonsurans* Malmsten), *T. rubrum Semon*, *Ctenomyces granulosum* Sab. and *Ctenomyces asteroides* Sab. He employed alanine and potassium nitrate as nitrogen sources and his results showed that all the fungi readily utilized alanine but none utilized the nitrate. Volkonosky also found that utilization of the carbohydrates in these fungi was extremely slow. He observed that the test fungus *C. serratus* did not ferment sorbite and the medium invariably became slightly alkaline.

Hawker (1936) and Schopfer and Blumer (1940) subsequently studied the nutrition of *Myxotrichum setosum* (Eidam) Orr and Plunkett, (= *Gymnoascus setosus* Eidam. (1963)). Hawker (1936) cultured *M. setosum* successfully in agar medium that contained 0·5% glucose, 0·35% KNO_3, 0·075% $MgSO_4$ and 0·175% KH_2PO_4. In her attempt to increase fruiting in this fungus, she experimented by adding to the above medium crude lentil extract or an inositol-free fraction of the crude extract. However, she found that the fruiting of the fungus did not show any remarkable improvement than what was obtained without the addition of the above extracts. Schopfer and Blumer (1940) reported that *M. setosum* grew well on a mineral dextrose medium which contained asperagine. The addition of thiamine or its intermediates had no effect on the growth of the fungus. This was mentioned by Robbins and Kavanagh (1942) who reviewed the vitamin deficiencies of the filamentous fungi.

Nyuksha (1953, 1955, 1957) studied *M. setosum* thoroughly and reported that pigmentation and growth occurred normally on sugars (feebly on lactose), starch and

cellulose but not on citric acid. The best source of nitrogen was peptone, potassium nitrate, asparagine and ammonium sulphate. He also reported that the fungus grew well on a medium which lacked a nitrogen source. This supports Berthelot's (1893) observation on a *Gymnoascus* spp. cited earlier. However, on such a medium no pigment was elaborated.

Physiological studies next gained momentum through the investigations conducted by Ghosh (1954, 1960), Kuehn and Crosby (1959, 1960), Kuehn *et al* (1962) and Kuehn and Orr (1962).

3.1 *Physiology as an aid to taxonomy*

Ghosh (1954, 1960) deviated from the early workers in carrying out a comparative nutritional study on carbon, nitrogen, vitamin and trace element requirements of some representatives of the Gymnoascaceae employing chemically defined synthetic media. Further, she compared her results with those of the Trichophytonae and the Aspergillaceae since these two families are taxonomically related.

The nutritional study undertaken by her was on the following fungi: *Arachniotus reticulatus* Kuehn, *Ctenomyces serratus* Eidam, *Eidaemella deflexa* (Berk) Benjamin, *Gymnoascus reessii* Baranetzky, *Myxotrichum uncinatum* (Eidam) Schroeter and, *Penicillium spiculisporum* Lehman. Sodium nitrate, ammonium nitrate, ammonium sulfate, asparagine, glycine, casein hydrolysate and an amino acid mixture were supplied singly as sources of nitrogen. All of the fungi grew on the organic nitrogen sources. The amino acid mixture was the best source for growth of *M. uncinatum, G. reessii, C. serratus* and *A. reticulatus*. Asparagine was the best source for *E. deflexa* and *P. spiculisporum*, although the latter grew on all the 7 sources of nitrogen tested. On the inorganic nitrogen sources tested sodium nitrate was assimilated only by *M. uncinatum* and *E. deflexa*. Glucose, fructose, galactose, maltose, sucrose, lactose, raffinose, xylose, dextrin and sodium lactate were the ten carbon sources which were tested. *Myxotrichum uncinatum* failed to assimilate only lactose, and grew best on raffinose, dextrin and sucrose. *Eidamella deflexa* used all carbohydrates but not sodium lactate. *Gymnoascus reessii* either did not grow at all or else grew only poorly on all the carbon sources tested. *Ctenomyces serratus* assimilated only fructose in that order. *Penicillium spiculisporum* used galactose, sucrose, xylose, glucose, maltose, dextrin and raffinose and failed to use lactose and sodium lactate. Thiamine, biotin, inositol, pyridoxine and calcium pantothenate were used in vitamin requirement studies. *Myxotrichum uncinatum* and *Eidamella deflexa* appeared to be autotrophic for all vitamins, *G. reessii* was heterotrophic for biotin and pyridoxine, *C. serratus* for thiamine and *A. reticulatus* for thiamine and pyridoxine. *Penicillium spiculisporum* showed a partial deficiency for pyridoxine and pantothenate. The trace elements which were tested included iron, zinc, copper and manganese. Probably only the results with *E. deflexa* are valid since the carbon and nitrogen sources were inadequate for *G. reessii, C. serratus* and *A. reticulatus*. Zinc and copper were essential for growth of *E. deflexa*. Manganese and copper seemed essential for growth of *P. spiculisporum*, while zinc was toxic.

This investigation of the nutritional requirements indicated the following:

(i) *Myxotrichum uncinatum* and *Eidamella deflexa* seem more closely related to each other on a nutritional basis than they are to *Arachniotus reticulatus, Ctenomyces serratus* and *Gymnoascus reessii.*

(ii) *Myxotrichum uncinatum* and *Eidamella deflexa* have nutritional requirements more similar to the *Aspergillaceae* than to the Trichophytoneae.
(iii) *Ctenomyces serratus*, *G. reessii* and *A. reticulatus* have similar nutritional requirements.
(iv) *Ctenomyces serratus*, *G. reessii* and *A. reticulatus* have nutritional requirements more similar to those of the Trichophytoneae than those of the Aspergillaceae.
(v) The nutritional requirements of *Penicillium spiculisporum* are very similar to those of other Aspergillaceae. Therefore, the present investigation supports the separation of this fungus from the Gymnoascaceae.
(vi) No clear cut physiological evidence is presented which would better support a classification which related the Gymnoascaceae more closely to the Aspergillaceae or to the Trichophytoneae than has been suggested by morphological studies.

However, this study gives additional support to the subsequent transfer of *E. deflexa* under the genus *Myxotrichum* by Orr *et al* (1963). This transfer was based on similar morphological characters and pigment elaboration by the two fungi. According to the authors reddish pigments which are produced in most of the culture media, to varying degrees, are probably characteristic of most species of *Myxotrichum*. They advocated more complete physiological comparisons in this direction. Kuehn and Crosby (1960a–d) and Kuehn *et al* (1962) provided a series of investigations on the growth and pigment elaboration of *Eidamella deflexa* (Berk) Benjamin, now *M. deflexum* Berk.

Earlier Kuehn and Crosby (1959) studied the nutritional requirements of *Pseudoarachniotus roseus* Kuehn. Optimum mycelial production was determined by employing 30 different carbon compounds and 43 different nitrogen sources which were supplied individually. Maltose, dextrin, xylose and soluble starch were the carbohydrates which permitted maximum growth while ammonium acetate and asparagine were the best nitrogen sources. These authors also studied the effect of pH and temperature on the growth of the fungus. The initial pH ranged between 6–9 while with optimum growth conditions the terminal pH was between 7 and 8. Using soluble starch as a carbon source and ammonium acetate as the nitrogen source, maximum mycelial weights were obtained in 32 hr at 37° C and in 88 hr at 28° C. They found that addition of low concentrations of Mn, Zn, Cu, Mb, Ca, Co, Cl, Fe and B to the basal medium did not stimulate mycelium production, but addition of 0·2 mg thiamine or 0·3 mg% biotin stimulated formation of mycelium.

Later, Kuehn and Crosby (1960a, b) worked out the carbon-nitrogen assimilation and some growth factor requirements of *Eidamella deflexa* (Berk) Benjamin (= *Myxotrichum deflexum*) in submerged cultures. They found that the nature of the nitrogen source affected the response of the fungus to a given carbon source and vice versa. Lactose, soluble starch, maltose and sorbitol were the carbohydrates which permitted maximum growth with peptone as a nitrogen source. Glycine was the best defined nitrogen source with lactose as the carbon source, while potassium nitrate was the best defined nitrogen source with soluble starch as a carbon source. Similarly, maltose was the best source with KNO_3. Mixtures of defined nitrogen sources do not permit greater mycelial production than glycine or KNO_3 alone. For *Eidamella deflexa* the initial favourable pH ranged between 6 and 8 with the optimum within 6·5 and 7. The maximum mycelial production resulted in 5 days at 20° C. In the same year they developed a synthetic medium for optimum growth and pigmentation of *Eidamella*

deflexa. The medium contained both 5% maltose and 5% glycine which were found essential to induce maximum pigmentation.

Kuehn and Crosby (1960c) and Kuehn *et al* (1962) while continuing their nutritional studies on *E. deflexa* investigated the rate of utilization of carbon and nitrogen and compared it with the rate of growth and pigment elaboration. They also studied the effect of varying concentrations of certain inorganic compounds on growth and pigmentation of *E. deflexa*.

Simultaneously, Kuehn and Crosby (1960d) studied the growth and nutrition of a strain of *Gymnoascus reessii* in shaken flask cultures and observed that the medium for optimum growth consisted of 5% maltose, 2% KNO_3, 0·2% K_2HPO_4, 0·2% $MgSO_4$, $7H_2O$, 1 mg% uracil, 1 mg% guanine, 5 mg% xanthine, 0·8 mg% biotin and 0·2 mg% pyridoxine. Optimum growth was obtained between a pH range of 5–8.

In the next series of work, Kuehn (1961,1962) studied the nutritional requirements of *Arthroderma tuberculatum* and *Myxotrichum uncinatum* and established two chemically defined basal media for the optimum growth of these fungi. In order to determine whether or not correlation exists between morphological variation and physiological characteristics, Kuehn and Orr (1962) again conducted a nutritional study of eight strains of *Gymnoascus reessii*. It was found that seven strains were similar in their assimilatory abilities, while one strain 0-309 (NRRL 3612), was consistently dissimilar from the others. They therefore opined that it could possibly be considered as a variety of *G. reessii*.

Nutritional and physiological studies on the dermatophytes continued to be thoroughly investigated (Stockdale 1953; Shome 1967). However, similar studies on members of the Gymnoascaceae suffered a set back for almost a decade subsequent to Kuehn's work in 1962. Ghosh and coworkers resumed the biochemical investigations and extended such studies further. Kashyap *et al* (1972) studied the nitrogen metabolism of *Arachniotus dankaliensis* (*Pseudoarachniotus roseus* Kuehn) in submerged cultures. They established the growth profile of the fungus in glucose-asparagine medium and studied the utilization of asparagine during different phases of growth by chromatographic analysis of amino acids in filtrate and in mycelium and of bound amino acids in buffer soluble proteins of mycelia. They found that the utilization pattern was well correlated with the growth profile of the organism. It was observed that an active conversion of asparagine into aspartic acid, glutamic acid, alanine, serine and glycine was manifested as growth proceeded. The latter amino acids were the principal constituents of the buffer-soluble proteins. Further tests pointed to a specificity in enzyme make-up. It was also observed that two different strains of *A. dankaliensis* (GR-1 and *P. roseus*) were closely similar and fairly stable. It was concluded that such metabolic specificity under a given condition could serve as a criterion for comparative studies.

3.2 *Saprophytic versus clinical behaviour*

Mohanty and Ghosh (1979) studied the nutritional biochemical behaviour of four strains of *Rollandina* Pat. in order to ascertain whether or not any nutritional parity exists between taxonomically related species and between strains of the same species isolated from saprophytic (*S*) and clinical (*C*) sources respectively. The fungi chosen were:

(i) *Rollandina capitata* Pat-S (GR-6, 0-112; = *Pseudoarachniotus marginosporus*),
(ii) *R. capitata*-C (GR-29, O-962, STM F 223; = *Pseudoarachniotus punctatus* Dutta

& Ghosh), (iii) *R. capitata*-S (GR-42, O-3540; type strain of *Narasimhella poonensis* Thirum and Mathur); (iv) *R. hyalinospora*-C (GR-52, O-1247 STM F 231; = *Pseudoarachniotus hyalinosporus* Kuehn, Orr & Ghosh). These are closely related fungi which posed taxonomic problems. Recently Roy *et al* (1978) placed these under the genus *Rollandina*. Morphological studies provided justification for acceptance of the three species (GR-6, GR-29, GR-42) as synonyms of *R. capitata*. Mohanty and Ghosh (1979) observed that the three strains of *R. capitata* behaved alike in respect to their RNA make up. *T* test conducted on DNA content of the mycelia/mg of dry weight supported the view that the four fungi belong to the genus *Rollandina*.

Physiological data of Mohanty and Ghosh (1979), showed differential behaviour of GR-6, and thus supported the observations of Roy (1978) made on its morphological characters; strain GR-6 thus distinctively stood apart from GR-29 and GR-42, as a variant. Further the quantity of dry weight and cellular protein was greater in saprophytic strains than those of the clinical strains at their respective peak periods of growth. The biochemical behaviour is genetically controlled and may have a bearing on taxonomy.

Scanning electron microscope studies carried out by Ghosh *et al* (1979) on ascospore morphology of the type strains of the four fungi used by Mohanty and Ghosh, have provided definitive evidence in support of positive relationships of these fungi. In their continued effort to clarify taxonomic confusions employing biochemical behaviour as a parameter,Mohanty *et al* (1983) reported a comparative study of amino acid metabolism of the same group of fungi studied by Mohanty and Ghosh (1979). In addition, the behaviour of the clinical strains was compared with that of saprophytic strains. Free and bound amino acids of the mycelia and free amino acids of the culture filtrates were analysed. Eighteen amino acids and two amides were detected. All four fungal strains were alike with respect to their amino acid composition at different stages of growth. Specific variations were noted between two species and among the clinical and saprophytic strains. Strain GR-6 is recognised as a variant strain of *R. capitata*.

In another work Ghosh and Sahu (1979) compared the growth pattern of *Rollandina hyalinospora* simultaneously in glucose-asparagine medium (Lilly and Barnett 1951) and in another with equimolar amount of ammonium acetate replacing asparagine. Good growth was observed in both. The authors suggested that ammonium acetate could be an useful and cheaper substitute for asparagine for routine cultivation of Gymnoascaceous members.

More recently, Kashyap and Ghosh (unpulished data) undertook the study of comparative physiology of some pathogenic members of the Gymnoascaceae. Two species of the genus *Pseudoarachniotus* Kuehn were chosen for this study. Each species had one strain isolated from clinical source and another from the saprophytic source. These were:

(i) *P. roseus* Kuehn (GR-1, saprophytic),
(ii) *P. roseus* Kuehn (O-3464, clinical),
(iii) *P. flavoluteus* Kuehn and Orr (GR-17-Saprophytic),
(iv) *P. flavoluteus* Kuehn and Orr (Gr-28, STM, F, 141, Clinical).

Orr *et al* (1977) pointed out that the above two species are closely related and at times caused difficulty in correct identification. The clinical strain GR-28 has since been transferred to *P. roseus* by them. Thus through this study an attempt was made to find out how far this transfer is physiologically and nutritionally justified. It was observed

that in general the saprophytic strains grew better in organic nitrogen sources and vice versa. This could reflect the difference in permeability characteristics of the organisms. In producing considerable amount of pigment, the saprophytic strain of *P. flavoluteus* (GR-17), stood apart from the other three strains, which were mild producers. Thus GR-28 was more akin to strains of *P. roseus*. In the above context it could be suggested that the strain GR-28 of *P. flavoluteus* is getting more adapted to its clinical (opportunistic) status and is therefore behaving more like a strain of *P. roseus*. It could possibly be at a transitional stage. This assumption is further supported by observing the growth profiles of the four strains.

Growth pattern of the stationary phase of *P. flavoluteus* -C(GR-28) closely resembled that of *P. roseus* -C(O-3464). Strain GR-1-S had a rudimentary lag phase in contrast to the other three strains. The nature of fundamental differences can only be indirectly spelled out. The difference lies in the nature of the relevant enzyme system. It may be said that these enzyme systems are constitutive in *P. roseus* -S(GR-1). In the rest of the cases the enzymes are adaptive and have to be induced possibly by the influence of the substrates themselves. The time taken by the other three organisms for inducing these enzyme systems necessarily slows down the process of building up of the amino acid pools required for the onset of active growth (Ghosh 1983).

3.3 *A numerical approach to the study of growth profiles*

The growth profiles of the strains of *Rollandina* as observed by Mohanty and Ghosh (1979), seemed to follow a regular pattern. This was the basis of motivation to undertake a theoretical study, in order to analyse the growth patterns quantitatively. The equation for the growth curve was obtained for each of the four species by Newton's forward and backward difference formulas. The rate of growth and the day on which the fungus is expected to attain maximum growth were predicted theoretically.

GR-52, GR-6, GR-29, were similar in the sense that their growth profiles fit a quadratic polynominal and their growth rate was a linear function of time. GR-42, was slightly different in that, its growth profile was found to fit a cubic polynomial and its growth rate was a quadratic function of time.

The relevance of undertaking such numerical methods is appreciated in view of the fact that, these predictions can give clues on certain taxonomical and behavioural parameters, which will finally help in a better planning of the actual experimental scheme (Ghosh and Ghosh 1983).

4. Conclusion

In presenting some of these physiological evidences, the author wishes to emphasise that metabolic patterns are intrinsically related to the nature of growth and ecology of the fungi. Further works in this direction are in progress to clarify the problems still existing in and around the family Gymnoascaceae.

Acknowledgement

The financial help from ARS USDA Grant no. FG-IN-626, project IN-ARS-206 and the supply of clinical strains from the School of Tropical Medicine (STM), Calcutta are gratefully acknowledged.

References

Ajello L 1962 Comments on the laboratory diagnosis of opportunistic fungus diseases; *Lab. Invest.* **11** 1033–1034

Apinis A E 1964 Revision of British Gymnoascaceae; *Mycol. Pap.* **96** 1–56

Ashbrook B M 1968 Isolation of perfect state of *Microsporum gypseum, Nannizzia incurvata* Stockdale 1967, from soil in north Carolina; *J. Elisha Mitchell Scient. Soc.* **84** 233–235

Arx J A von 1971 On *Arachniotus* and related genera; *Persoonia.* **6** 371–380

Baranetzky J 1872 Entwicklungsgeschichtedes *Gymnoascus reessii*; *Bot. Zinct.* **30** 145–160

Benjamin R K 1956 A new genus of the Gymnoascaceae with a review of the other genera; *El. Aliso* **3** 301–328

Castellani A 1937 A preliminary report on two pathogenic fungi; *Trichophyton dankaliensis n. sp.*; *Sporotrichum anglicum n. sp*; *J. Trop. Med. Hyg.* **40** 313–318

Dawson C O and Gentles J C 1961 The perfect states of *Keratinomyces ajelloi* Vanbreuseghem, *Trichophyton terrestre* Durie and Frey and *Microsporum nanum* Fuentes; *Sabouraudia* **1** 49–57

Delamater E D 1937 *Eidamella spinosa* Matruchot and Dassonville refound; *Mycologia* **29** 572–582

De Vroey Ch 1966 Etude mycologique de Gymnoascaceae isolees de lesions cutanees et de la terre en Republique de Somalie; *Ann. Soc. Belge. Med. Trop.* **46** 457–464

De Vroey Ch 1970 Contribution a letude des Dermatophytes et dautres Gymnoascaceae; *Ann. Soc. Belge. Med. Trop.* **50** 1–174

Emmons C W 1954 Isolation of *Myxotrichum* and *Gymnoascus* from the lungs of animals; *Mycologia* **46** 334–338

Emmons C W 1962 Natural occurrence of opportunistic fungi; *Lab. Invest.* **11** 1026–1032

Fennell D I 1973 In *The fungi: An advanced treatise* (New York, USA: Academic Press) Vol 4A. 45–64

Ghosh G R 1954 *Comparative physiology of some representatives of the Gymnoascaceae* Ph.D. thesis. University of Illinois. USA

Ghosh G R 1960 Comparative physiology of some representatives of the *Gymnoascaceae*; *Mycopathol. Mycol. Appl.* **13** 161–180

Ghosh G R, Sur B and Roy K 1976 Dermatomycoses in Cuttack, Orissa and a report on pseudocleistothecia formation by strains of *Trichophyton rubrum* Castellani; *Kavaka* **4** 25–30

Ghosh G R and Sahu M 1979 Comparative physiology of *Rollandina hyalinospora* in glucose asparagine and glucose-ammonium acetate media; *Indian J. Bot.* **2** 148–155

Ghosh G R, Orr G F, Pier A C and Gallagher J E 1979 Comparative ascospore morphology of certain strains of *Rollandina* by scanning electron microscopy; *Mycotaxon* **10** 21–28

Ghosh G R 1983 Studies on pathological behaviour of Gymnoascaceae; *Kavaka* **11** 11–20

Ghosh G R and Sarangi Shashimani 1983 Survey of Keratinophilic fungi inhabiting *Passer domesticus* in Orissa (India) IMC3 Abstracts 456

Ghosh S and Ghosh G R 1983 A numerical approach to the study of growth profiles of certain members of Gymnoascaceae by the method of polynomial Interpolation. IMC3 Abstracts 457

Griffion D M 1960a Perfect stage of *Microsporum gypseum; Nature (London)* **186** 94–95

Griffion D M 1960b The re-discovery of *Gymnoascus gypseum* and a note on *Trichophyton terrestre*; *Trans. Br. Mycol. Soc.* **43** 637–641

Hawker L E 1936 The effect of certain accessory growth substances on the sporulation of *Melanospora destruens* and of some other fungi; *Ann. Bot.* **60** 699–717

Kashyap R S, Biswas S B and Ghosh G R 1972 Nitrogen metabolism of *Arachniotus dankaliensis* in submerged culture; *Mycologia* **64** 727–736

Kuehn H H 1955a Observation on Gymnoascaceae I. *Myxotrichum uncinatum* and a new species of *Myxotrichum*; *Mycologia* **47** 533–545

Kuehn H H 1955b Observation on Gymnoascaceae II. Two new species of *Myxotrichum*; *Mycologia* **47** 878–890

Kuehn H H 1957 Observations of Gymnoascaceae IV. A new species of *Arachniotus* and a reconsideration of *Arachniotus trisporus*; *Mycologia* **49** 55–67

Kuehn H H and Crosby P F 1959 Growth and nutrition of *Pseudoarachniotus roseus* in submerged culture; *Mycopathol. Mycol. Appl.* **11** 109–127

Kuehn H H and Crosby P F 1960a Nutritional studies of *Eidamella deflexa*. I. Carbon-nitrogen assimilation and some growth factor requirements; *Mycopathol. Mycol. Appl.* **12** 145–155

Kuehn H H and Grosby P F 1960b Nutrition of *Eidamella deflexa* 11 Development of a synthetic medium for optimum growth and pigmentation; *Appl. Microbiol.* **8** 133–135

Kuehn H H and Crosby P F 1960c Nutrition of *Eidamella deflexa* III. Further studies on carbon and nitrogen assimilation and pigment synthesis; *Appl. Microbiol.* **8** 148–159

Kuehn H H and Crosby P F 1960d Growth and nutrition of a strain of *Gymnoascus reessii* in shaken flasks; *Mycopathol. Mycol. Appl.* **12** 349–359

Kuehn H H 1961 Nutritional requirements of *Arthroderma tuberculatum; Mycopathol. Mycol. Appl.* **14** 123–128

Kuehn H H 1962 A preliminary study of the nutritional requirements of *Myxotrichum uncinatum; Mycopathol. Mycol. Appl.* **16** 222–228

Kuehn H H, Crosby P F and Phillips R F 1962 Nutrition of *Eidamella deflexa*. IV. Rate of utilisation of sugar and amino nitrogen compared with rate of growth and pigment elaboration; *Mycopathol. Mycol. Appl.* **17** 219–224

Kuehn H H, Orr G F and Ghosh G R 1961 A new widely distributed species of *Pseudoarachniotus; Mycopathol. Mycol. Appl.* **14** 215–229

Kuehn H H and Orr G F 1962 A nutritional study of eight strains of *Gymnoascus reessii*; *Mycopathol. Mycol. Appl.* **16** 351–361

Kuehn H H, Orr G F and Ghosh G R 1964 Pathological implications of Gymnoascaceae; *Mycopathol. Mycol. Appl.* **24** 215–229

Kwon-Chung K J 1972 *Emmonsiella capsulata*: Perfect state of *Histoplasma capsulatum*; *Science* **177** 368–369

Latham M E 1909 Nitrogen assimilation of *Sterigmatocystisnigra* and the effect of chemical stimulation; *Bull. Tor. Bot. Club.* **36** 233–244

Lilly V G and Barnett H 1951 *Physiology of fungi* (New York: McGraw Hill) 1–464

Matruchet L and Dassonville C 1899 sur le *Ctenomyces serratus* Eidam. Compare sux Champignous des Teignes; *Bull, Soc. Mycol. Pr.* **15** 305–310

Matruchet L and Dassonville C 1900 Sur une forme de reproduction d'order eleve chez les *Trichophyton; Bull. Soc. Mycol. Fr.* **16** 201–208

Matruchet L and Dassonville C 1901 *Eidamella spinosa* dermatophyte prednisant desperitheces; *Bull. Soc. Mycol. Fr.* **17** 123–132

Mc Donough E S and Lewis A L 1968 The ascigerous stage of *Blastomyces dermatitidis; Mycologia* **60** 76–83

Mohanty R K and Ghosh G R 1979 Comparative physiology of some strains of *Rollandina; Mycotaxon* **10** 29–36

Mohanty R K, Ghosh G R and Biswas S B 1983 Comparative physiology of some strains of *Rollandine* II. Amino acid metabolism; *Mycotaxon* **17** 364–376

Novak E K and Galgoczy J 1966 Notes on dermatophytes of soil origin; *Mycopathol. Mycol. Appl.* **28** 289–296

Nyuksha Y P 1953 A physiological study of the fungus *Gymnoascus setosus* Eidam. I; *Microbiologia* **22** 15–22

Nyuksha Y P 1955 Some reasons for spoilage of books; *Nature (Moscow)* **44** 94–96

Nyuksha Y P 1957 A physiological study of the fungus *Gymnoascus setosus* Eidam. II; *Microbiologia* **20** 309–315

Orr G F, Kuehn H H and Plunkette O A 1963 The genus *Myxotrichum* Kunze; *Can. J. Bot.* **41** 1457–1480

Orr G F, Ghosh G R and Roy K 1977 The genera *Gymnascella, Arachniotus* and *Pseudoarachniotus*; *Mycologia* **69** 126–163

Rioux J A, Jarry D M and Jarry D T 1966 *Ctenomyces, Arthroderma* on *Trichophyton* find une controverse et nouvelle acceptation dee terme de Dermatophytes; *Ann. Parasitol. Hum. Comp.* **41** 523–549

Robbins W J and Kavanagh V 1942 Vitamin deficiencies of the filamentous fungi; *Bot. Rev.* **8** 411–471

Roy A and Chunder M 1963 *Pseudoarachniotus* sp. as the etiological agent of a case of human mycosis; *Bull. Cal. Sch. Trop. Med.* **11** 108–109

Roy K, Ghosh G R and Dutta S K 1972 Keratinophilic fungi and the prevalence of dermatomycoses in Orissa, India; *Sabouraudia* **10** 218–229

Roy K, Ghosh G R and Orr G F 1978 The genus *Rollandina. In Proc. Int. Sym. on Taxonomy of Fungi*, held on January, 1973. Madras, India Part I: 215–244

Roy K 1978 *Studies on Gymnoascaceae and related fungi* Ph.D thesis. Utkal University

Schopfer W H and Blumer S 1940 Recherches sur la repartition de l'heterotrophie par rapport a l'ankurine chez les champignons; *Arch. Microbiol.* **11** 205–214

Schroeter J 1893 Die Pilze Schlesiens In Cohn; *Krypt. Fl. Schles.* **3** 210–213

Shome S K 1967 Dermatophytes—their physiology and nutritional requirements; *Bull. Natl. Inst. Sci. India* **35** 65–78

Sigler L, Gaur P K, Lichtwardt R W and Carmichael J W 1979 *Renispora flavissima*, a new gymnoascaceous fungus with tuberculate. chrysosporium conidia; *Sabouraudia* **8** 85–162

Stockdale P M 1953 Nutritional requirements of the dermatophytes; *Biol. Rev.* **28** 84–104

Stockdale P M 1961 *Nannizzia incurvata* gen. nov. sp. nov. a perfect state of *Microsporum gypseum* (Bodin) Guiart Grigorakis; *Sabouraudia* **1** 41–48

Sur B and Ghosh G R 1980 Keratinophilic fungi from Orissa, India; Isolation from soils; *Sabouraudia* **18** 269–274

Vanbreuseghem R 1973 Fungi as pathogens; *Clin. Excerpts* **35** 1137–1143

Volkonosky M 1934 Sur la nutrition de quelques champignons saprophytes et parasites. *Ann. Inst. Pasteur* **52** 76–101

Young C N 1968 Pseudocleistothecia in *Trichophyton rubrum*; *Sabouraudia* **6** 160–162

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 209–227.
© Printed in India.

Conidia as modified hyphae

E DESCALS*

Department of Biological Sciences, University of Exeter, England

* Present address: Cases Noves, Esporles, Mallorca, Spain

Abstract. An attempt is made at a unified morphological analysis of the hyphomycetous anamorph as seen in its two phenotypic expressions: the vegetative (= hyphae and mycelia) and the reproductive (= conidiophores, conidiogenous cells, proconidia and free propagules). (Multi-hyphal structures are ignored). A distinction is made between cytological and structural criteria. The basic units used are *cells* (or compartments), and their assemblage into *hyphae*, and at a higher level of organization, *organs*. The description of *elements* (= the simplest recognisable structures) is suggested for cases where compartments are absent or poorly defined. Eight independent characters are proposed for the description of the mature anamorph. A unified approach is also suggested for the study of the development of the anamorph. Basic, general processes are growth (= different combinations of elongation, branching and transformation), maturation, lysis and secession (as defined in this paper), and, in the case of recognizable compartments, cell division (= septation). These processes, when integrated in various specific sequences, result in well known patterns, seen in both vegetative development and asexual reproduction. Examples are drawn from species with ameroconidia, more intensively studied in recent literature, as well as on lesser known species with simple, septate, or compound conidia. Some criteria are proposed for an ordered observation of independent processes which could provide a basis for a more stable descriptive terminology.

Keywords. Hyphal morphogenesis; conidiogenesis; aquatic hyphomycetes; taxonomy.

1. Introduction

The descriptive terms on which anamorph taxonomy relies may refer to any combination of morphological criteria (= structural or developmental) at light and electron microscope levels, as well as functional. The choice of structures described is arbitrary, the conidiogenous cell and proconidium taking precedence over the conidiophore; and the mycelium, also a component of the anamorph, being ignored, probably because it is assumed to be structurally unspecialised and developmentally plastic. Terms may refer to the mature structure of the components of the conidial apparatus (*e.g.* 'scolecoconidium", or "macronematous conidiophore"). Secondly, the ways by which that structure is obtained (ontogeny) are expressed with terms describing complex processes producing a plurality of conidia on specialized structures (*e.g.* phialides, annellides, sympodulae . . .). Some of these processes have never been defined accurately, or are the source of great confusion, due to the intergradation or partial overlap of their component processes. A less ambitious but increasingly popular approach describes simpler processes, such as the "porogenous initiation" of conidial primordia, or conidial "secession by schizolysis". Minter *et al* (1983), following the latter trend, and concentrating on the conidiogenous cell, have recently proposed a further category of terms, based on the light- or electron microscope observation of processes such as changes of external shape, septation, wall building and lamination, and separation of resultant layers (*e.g.* "initiation", "maturation" and "delimitation" of proconidia, "proliferation" of conidiogenous cells and "regeneration" of the coni-

diophore apex through a spent cell). They also indicate that these terms may be applicable to other parts of the conidial apparatus. Nevertheless, they imply that there may still be problems in the recognition of *overlapping processes* (such as "proliferation" and conidial "maturation") in *Basipetospora rubra* (where, in our view, one of these terms is superfluous), or of *intergrading processes*, such as "proliferation" and "initiation" in *Belemnospora epiphylla*, where we are forced into arbitrary spatial delimitations (*i.e.* "proliferation refers to events occurring near the base of the cell, and initiation to those near the apex"). Another difficulty lies in the application of the term "delimitation" to cases of "integrated conidiogenesis" (see below), *i.e.* the development of the initial (= first-formed) apical, integrated, proconidia on undifferentiated conidiogenous cells, so common among the Ingoldian anamorphs (= which include many aquatic hyphomycetes). If the primordium cannot be easily delimited, neither can the conidiogenous cell, and this may also lead to redundant terminology. This paper will attempt to reduce ontogeny to processes so simple that they should behave independently from each other and thus become convenient "building blocks" with which to assemble the seemingly intricate patterns of conidiogenesis.

It has become quite obvious that branched conidia of Ingoldian anamorphs can be very much like branched hyphal systems, differing only in their specialized sites of secession (and in lesser characters affecting shape, etc.) (*e.g. Varicosporium delicatum*, figure 6). Having in the recent past examined some of the literature on hyphal morphogenesis, this similarity between vegetative and reproductive structures can be attributed to their common developmental processes. A structural continuum can be easily assembled, with species like the above at one end, leading on to others with conidial branches becoming progressively shorter, until only one-celled, and ending with broad-based outgrowths on simple (= unbranched) conidia. From here it is only a matter of smoothening out conidial outlines to achieve complete reduction to spherical conidia. (This process would require a parallel regression of septation from multi- to uni- to aseptate branches, then on to dictyo-septate (already unbranched), phragmo-, didymo- and aseptate conidia). Expressing this range of structure even more succinctly one could say that secession has simply displaced itself along mycelial branches of progressively lower order, finally ending at the base of the apical cell. As a link can thus be established between hyphal morphogenesis and conidiogenesis, it should be possible to propose common descriptive terms for both the structure and development of the whole of the anamorph. Clutterbuck (1970) states that "many cell functions are operative in both hyphal and conidiating (= sporulating) states rather than there being a separate set of genes governing metabolic processes in the two phases of the life cycle" and that "relatively few genes are confined in their function to one phase of the life cycle", as evidenced by cases in which mutations causing defective conidiation can also affect growth processes of vegetative hyphae (and of some of those in sexual reproduction). Thus there seems to be a genetic basis for this unified approach. Our second aim then will be to apply to conidiogenesis criteria used in the description of hyphal morphogenesis. These are either cytological (involving cell, or septation, cycles) or structural (referring to changes of hyphal shape such as tip growth and branching). Prior to a discussion on ontogeny, we need to propose a few terms for describing the *mature structures* of the anamorph. Secondly, a further set of terms, representing simple *developmental processes*, will be discussed (ignoring "multihyphal structures" (Griffin 1981), formed by aggregation or cohesion mechanisms, which are outside the scope of this brief essay).

2. Structural components of the anamorph

Because septa are often not clear, (*i.e.* cytological criteria cannot be used), we may instead resort to structural criteria only, and describe the simplest possible recognizable outlines or shapes of the anamorph (called here "*elements*"), and their arrangement into *organs*. Elements can also be defined as entities resulting from a single, uninterrupted process of elongation, branching or transformation (see below). The undifferentiated mycelium consists of only leading and branch hyphae, in its simplest case. On the other hand, the conidial apparatus, after having completed all its functions, may consist of a host of different elements, which have been diagrammed in figure 1. These could be: the conidiophore axis, each of its branches of various orders, recognisable conidiiferous cells (when describing mature structures, the term "conidio*genous* only implies an ontogenic function), each of the elements of a compound proconidium, any elements

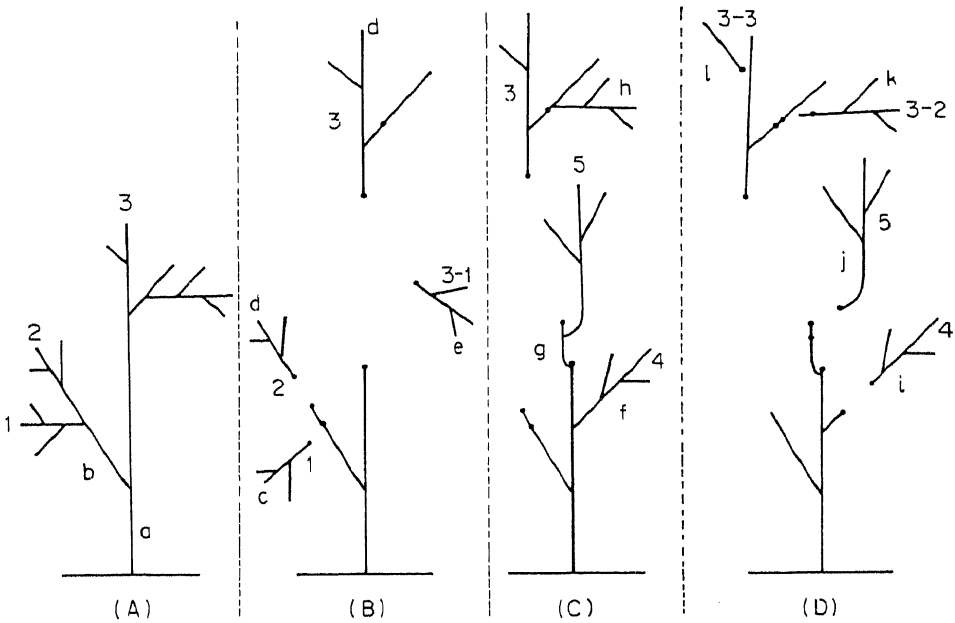


Figure 1. Components of a conidiogenous structure. (Primary propagules are numbered from 1–5. Secondary propagules are represented by the second set of numbers, *e.g.* "3–3" in figure 1D represents one of three propagules derived from the primary conidium labelled "3" in figure 1B. In this diagram processes of lesser importance *e.g.* septation and transformation, have been omitted for the sake of simplicity.) **A.** Mature conidiophore prior to secession. Notice the structural similarity with a vegetative mycelium. **a.** conidiophore axis, **b.** conidiophore branch. **B.** the same conidiogenous structure after secession (the sites of secession are indicated with black circles). **c.** a lateral primary conidium; **d.** apical conidia. Notice that elongation of branches has taken place *after* secession, and that part of conidium 3 has also seceded (**e**). **C.** regrowth of the conidiogenous structure. **f.** New conidiophore branch ending in a terminal conidium, **g** a series of two sympodial (regrowth) branches (the terminal conidium produced on the first branch **g.** has been omitted). Another conidium terminates the second branch (**j**) **i.** **h.** a secondary conidium is produced directly on conidium 3. **D.** A later stage in sporulation. **i.** the second-generation primary conidium has seceded; **j.** the second sympodium has also seceded, but not its terminal conidium yet; **k.** secession of the secondary conidium; **l.** secession, followed by elongation of a single conidial branch.

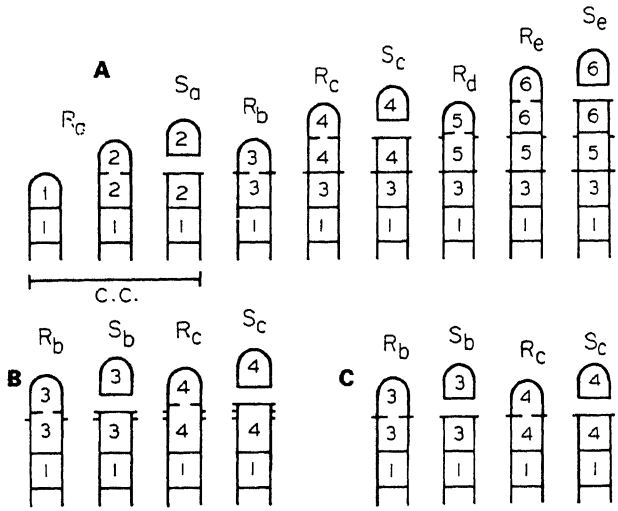


Figure 2. Diagram of modes of conidial production with monopodial regrowth. (Numbers represent cell generations). **A.** alternate conidiogenous cycles, resulting in seriate "conidiogenous cells" nos. 3, 5 and 6. The first three figures represent one conidiogenous cycle, made up of a duplication cycle followed by secession. **B, C.** consecutive conidiogenous cycles (the first conidiogenous cycle is omitted, as it is identical to that in **A. B.** Annellide. **C.** Apical-growth phialide. Consecutive secessional septa are deposited at the same level.

resulting from post-secession conidiophore branching (called by some "proliferations", but see below), and finally free structures (= naturally seceded) such as conidia, parts of these (= part-conidia), ramoconidia, caducous conidiophore branches, and any further elements inserted on these. We define *compound* conidia as those with more than one element, and *simple* conidia as those with only one. This way, we avoid arbitrary terms such as "staurospore" sensu Kendrick and Nag-Raj (1979) based, for example, on relative length of branches.

Ellis (1971) proposed the terms "integrated" *vs* "discrete" for conidiogenous cells. Two characters were applied: *orientation* with respect to the subtending element, (= "integrated conidiogenous cells are incorporated in the main axis . . . ") and *shape* (= "discrete conidiogenous cells often have distinctive shape . . . "). This leads to confusion, because a cell could, for example, be distinct and also incorporated along the main axis. In this paper, the terms are used in the latter sense only (= applying a structural criterion), and used for any element of the anamorph.

Adapting Stearn's (1973) botanical terminology to fungi, we see that any element can be described with the following "*individual absolute characters*", (based on structural criteria only): (i) *Form* (or shape), or, if this is projected onto one plane, outline, of which there may be more than one, (ii) *size* (used only to distinguish species or lower taxa), (iii) *wall and/or protoplasmic characteristics*, again of lesser taxonomic importance. "*Individual relative characters*" are (iv) *insertion* (or *location*) (with respect to its subtending element), and (v) *direction* (or *orientation*) (either with respect to the subtending or to its same-order (or sister) elements). If the structure described is an organ we also use "*collective characters*": (vi) *arrangement* of elements with each other or in relation to their subtending element, and (vii) their *number*, usually variable.

3. Ontogenic terms

Proliferation needs defining at this stage. It has been used as a compound term in the sense of regrowth of the conidiogenous cell after secession, giving rise to either new cells (a cytological criterion) or to mere extensions of the original conidiogenous cell (a structural criterion). However, it has been used in a purely structural sense by Berkeley (1849, in Hughes (1951)) when referring to "proliferous conidia" and also by other authors for other structures. We will therefore apply it to any element or structure of the anamorph, when it reproduces itself or multiplies one or more times. Another term which needs defining is "*growth*". It can be a process based on physiological criteria. Prosser and Trinci (1979) apply it to events in parts of the hypha which are not elongating, but synthesising building materials for the elongating apex. Even if used in a purely morphological sense, it is a compound term which may involve more than one process (*e.g.* elongation, branching and transformation), leading ultimately to enlargement. It can also be used in a cytological sense, when applied to dividing cells.

3.1 *The mycelium*

The following discussion is a summary of hyphal morphogenesis taken from the literature.

Mycelial growth at its simplest is by a highly organized combination of *apical elongation* and *branching*. Higher fungi integrate *septation* (= primary) into this process, in a more or less well-ordered sequence depending on the species (and more specifically, on cell nuclear number). Hyphal portions at rest, or not subject to the apical dominance of growing hyphal tips, or which may find themselves again at the apex by damage or by disjunction, are capable of resuming growth (= *regrowth*). Other processes may be recognizable, i.e. *transformation* (literally: change of form of cells or elements, also known as "differentiation", a term which may, however, apply to physiology or other aspects of growth), and *maturation*. Higher fungi also undergo frequent *anastomoses* of branch hyphae, which can be viewed as the result of elongation (with a high degree of tropism) followed by outer wall dissolution. Other *lytic processes* can be important in fungi (*e.g.* in dolipore septum dissolution in basidiomycetes) and culminate in highly specialized modes of *secession*, where lysis is either limited to septal fission (= schizolysis) or it involves cytoplasmic degradation followed by outer wall lysis (= rhexolysis).

Hyphal *elongation* is normally apical, (figure 3), (and is assumed, sometimes maybe wrongly, to occur only by apical wall building). Apical elongation is constant and indefinite under stable and favourable conditions. Hyphal orientation is affected by two factors. One is genetic control, by which for instance patterns of branching with characteristic angles are fixed. Another example would be "spiral growth" (Trinci 1984). The second factor is environmental control (*e.g.* nutrients, light, water etc . . .). Elongation can be independent of *septation* (Trinci 1978), as seen in most lower fungi. Septation may follow at a constant distance from the apex (= "progressive septation"), as seen in uninucleate cells of *Basidiobolus ranarum* and in basidiomycetous monokaryons (where nuclear and cell cycles are synchronized); or it can happen in successive waves of deposition ("multiple septation") as in multinucleate cells of *Neurospora* and *Aspergillus* (Clutterbuck 1970; Fiddy and Trinci 1976) (figure 3). Septation taking place in any elongating cell, whether apical or intercalary, is called *primary*, and that in older,

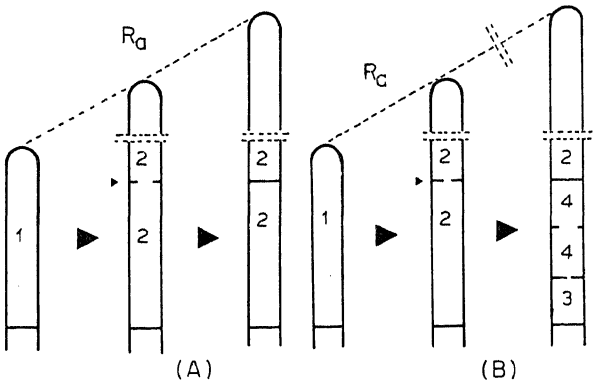


Figure 3. Two basic types of hyphal replicative growth, (adapted from Fiddy and Trinci 1976), presumably applicable to monoblastic conidiogenesis. **A.** Progressive septation, whereby septa are laid down in an ordered progressive series, (as seen in species with uninucleate cells). **B.** Multiple septation. Septa can be laid down in disordered sequences (as seen in species with multinucleate cells).

elongated cells, *secondary*. This may also occur in apical or intercalary cells. In hyphae growing at a linear rate, primary septation results in daughter cells of the same, (= characteristic) length as the mother cell. Secondary septation results in much shorter daughter cells.

Hyphal growth in its simplest form is therefore an uninterrupted series of duplication or *septation cycles* (figure 2) comparable to the cell cycles of unicellular organisms (bacteria and yeasts). It has also been appropriately called replicative or replicatory growth (Prosser and Trinci 1979; Smith 1978). An important fact to remember is that, from the cytological point of view the last two cells of any apically elongating hypha are always sister cells. This will have implications in the analysis of conidiogenesis.

Branching starts as a localized cell transformation, but then proceeding as unrestricted elongation. If it occurs in advancing portions of the mycelium it is integrated with apical elongation, producing characteristic patterns with constant "length and branch ratios" (Prosser 1979). The most common one is ***monopodial*** with progressive alternate development of branch hyphae on a leading hypha or "monopodium". Lateral branching may occur from the apical cell, or it may be dependent on septation, often being initiated from immediately below septa. Apical branching, resulting in dichotomies or coronate arrangements, is relatively uncommon, but can be a normal feature of some species too (Trinci 1978), or it can occur as a response to submersion, as in *Fusarium* (Robertson 1958, 1959).

Regrowth may take place either at newly constituted apices after natural secession or cell damage, or in older portions of hyphae not subject to apical dominance, and thus capable of resuming physiological polarity. The latter is seen in "sympodial branching" of a mutant of *Neurospora crassa* (Trinci 1984). In the former, regrowth may occur by elongation after softening of an end septum or half septum (depending on whether it has been preceded by schizo- or rhexolysis), (*e.g.* intrahyphal hyphae (or endohyphae) a common feature in fungi) (figure 4). Regrowth may also be initiated by branching from a region adjacent to the old apex, the resulting arrangement called sympodial too (figure 4).

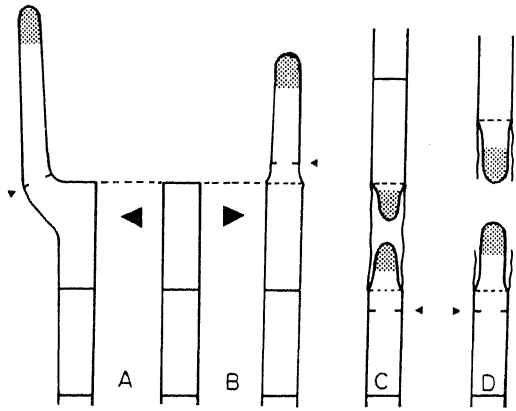


Figure 4. Diagram of types of regrowth. **A.** Sympodial. **B, C, D.** Monopodial. Notice that both cases fundamentally consist of replicative growth. (Small arrowheads show primary septation) **C.** Intrahyphal hyphae growing in lysed cell. **D.** Rhexolytic secession, as seen in *Anguillospora* (figure 12) (dotted lines within cells indicate secessional scars).

"Ring (or intercalary) elongation", (involving only surface increase of lateral walls), a component of a third type of regrowth, can be recognized with the light microscope by direct observation of forward displacement of septate hyphae closer to the apex. (Also special cytological techniques are available, such as immunofluorescence). The process would only be advantageous in aerial or aquatic hyphae. Assimilative hyphae lying on a solid substratum, if they were to grow by intercalary elongation, would have to drag themselves on the underlying surface. Ring elongation is not reported in the literature surveyed on hyphal morphogenesis.

It must be emphasized that the actual site of any type of elongation cannot be detected with the light microscope alone, as it may occur diffusely or in more or less localized regions of wall building. All we are allowed to describe, therefore, is a change in total length of a cell or element. Therefore Minter and co-workers' three types of wall building must be used with well defined criteria. In developing basidiospores of *Coprinus*, for example, the electron microscope has proven the successive appearance of wall building in different sites (apical, followed by "band" growth) in what appears to be a simple blastic process (McLaughlin 1977).

Hyphal transformation is a limited and relatively irreversible growth process, not to be confused with temporary swelling and constriction due to elasticity of unset walls, as seen, among other cases, in developing basidiospores (Corner 1948, in Madelin 1981). Transformation may affect individual cells or parts of cells, or, by intercellular integration, a whole multiseptate element (*e.g.* fusoid septate portions of hyphae with a predetermined shape). It involves widening or attenuation, or combinations of both. There is always growth, however, and attenuation is only taken in a relative sense, *i.e.* an element becomes narrower (as it elongates) in relation to its previous shape, although the overall structure continues widening. Widening may take place jointly with elongation, at the apex, or at a later stage more diffusely and independent of elongation (*i.e.* by Minter *et al* (1983) diffuse wall building). Attenuation, however, seems to occur only at the elongating tip (see under "proconidium") (figure 5).

Maturation is here used in its generally accepted sense of ageing, *i.e.* gradual alterations of structure or ornamentation, rigidity or pigmentation of walls, or of

vacuolization, droplet formation, pigmentation, etc., of protoplasm. Here again the process may affect one or more cells, or even part of a cell. It needs no further discussion here, as it is unimportant in this study of basic morphological processes.

In an unfavourable environment, or in aged mycelia, or maybe even as part of a process of unspecialized propagation, hyphae may separate (= *secession*) naturally by fragmentation (septal fission) or presumably also by lateral wall fission or breakage with or without cell autolysis.

Hyphal *anastomosis*, though a biologically important process, will not be discussed here, as it has few analogies in conidiogenesis worth mentioning. Exceptions would be conidia of *Clathrosphaerina* (Beverwijk 1951), *Isthmospora* (Hughes 1953), and *Riessia semiophora* (Barron 1972) among others.

3.2 *The conidiogenous cell*

3.2a *Replicative growth*: The development of only ameroconidia from monoblastic conidiogenous cells has been the basis for most of the important terminology on conidiogenesis. The key observation here is that a cell undergoes a "conidiogenous cycle" (Hammill 1981) equivalent to a hyphal duplication cycle, followed by secession (figure 2). As in hyphae there is *elongation* of the conidiogenous cell, presumably but not necessarily by apical wall building. This soon leads to primary *septation* (Minter and co-workers' "delimitation"). The distal daughter cell resulting from primary septation becomes the proconidium. The proximal cell (its sister cell), after secession, remains attached to the thallus. It may be referred to as the conidiiferous cell (Barron 1972) but not conidiogenous, unless it is involved in further conidiation cycles. Conidiogenesis with elongation is known as "blastic" (Kendrick 1971, and Cole and Samson 1979).

"Delimitation" (= the laying down of the basal septum) deserves some consideration at this stage. The term is only useful, in a physiological sense, if it involves complete severance of cytoplasmic continuity, which would force the resulting conidium into an autonomous existence. This is not always the case, as seen in time-lapse cinemicrography of *Actinospora* (Webster *et al* 1975). Using structural criteria we have two alternatives. If the proconidium is *discrete*, whether it is septate or not, delimitation will result in an abrupt change of shape separating it from the subtending element. (This may happen well before basal septum completion). If the proconidium is *integrated* there is no precise delimitation. Using cytological criteria, delimitation would be the full deposition of a basal septum (perforate or not). This can only be recognised at the base of initials of ameroconidia, where there are no more septa to be laid down. If septate proconidia show multiple septation there should be a time lapse between the appearance of the basal septum and the remaining septa, in which case the basal septum could be detected. It is also possible that the basal septum is structurally recognizable from the rest, as claimed by some. If proconidia display progressive septation, delimitation may again be unrecognizable, (= the proconidium and conidiiferous cell are indistinguishable). It is obvious from the above that, whichever morphological criterion is used, there will be cases when "delimitation" is not applicable. Therefore the term at the moment is only of relative value. It should be interesting to study correlations between septation processes in proconidia and their respective mycelia. However, they need not follow the same pattern. In *Aspergillus*, the mycelium replicates by multiple septation, but its conidiogenesis (from "apical-growth phialides") is strictly by successive replications.

3.2b *Branching*: As in hyphae, branching may be closely integrated with the conidiogenous cycles although obscured by transformation processes. If these are ignored, any form of polyblasty can be interpreted as a simple process of cellular branching (*e.g.* botryoblastospore formation), either synchronously or sequentially, the choice probably again depending on the nuclear number. (It is obvious that a uninucleate cell cannot undergo synchronous sporulation). Although starting from a minute pore, botryoblastores, for example, may lay down a primary septum too, and eventually secede from there.

3.2c *Regrowth*: If blastic conidiogenesis is "indeterminate" (Sutton 1980), that is, if apical dominance is recovered after secession, we have regrowth. This means that a new fertile portion is formed by a simple growth process of duplication, and will always involve at least one duplication. This sequence may repeat itself indefinitely. Several patterns can develop which will be briefly outlined.

If regrowth is by elongation, (= monopodial) (called "percurrent" by others because the new apex grows through an annular scar, which is not always visible) there are three possibilities: (a) if the next secessional septum is laid down at the same level as the initial scar (as seen with the light microscope) we have "apical-growth phialides", (a term based on Minter and co-workers' distinction between phialides with replacement and ring wall building) (figure 2). If repeated, there is an accumulation of progressively narrower scars, called the periclinal thickening, and visible with phase microscopy. It is a case of *consecutive conidiogenesis*. (b) A slight variant of this is if consecutive secessional septa are laid down in a close progressive series, resulting in annellides. This sequence is also one of consecutive conidiogenous cycles (figure 2B). It is evident that in "apical-growth phialides" and annellides there is no conidiiferous cell proliferation. There are cases in which the electron microscope shows new primary septa below the original conidial scar, and thus hidden within the collarette (Minter *et al* 1983). But, fundamentally, the pattern remains one of consecutive conidiogenous cycles. We must remember that, as growth is a component of any blastic conidiogenous cycle, the pattern of conidiogenesis can be affected to a significant extent by environmental conditions. These could induce unequal divisions in hyphal growth (= differences in length between sister cells), which, in conidiogenesis would result in septal deposition at sites higher or lower than the previous secessional scar. (c) If two primary septa are deposited consecutively, and only the second undergoes fission, a new vegetative cell is laid down above the original conidiogenous cell (the conidiiferous cell proliferates in this case). Conidiogenesis thus skips one duplication cycle. If this process of *alternate conidiogenesis* is repeated we can see series of cells being laid down progressively (called by some seriate conidiogenous cells improperly because they have never been fertile). (Another less likely possibility is that consecutive vegetative cells are the result of secondary septation. This would produce cells of half the characteristic length, unless there is ring growth. But secondary septation appears to occur mostly in resting portions of hyphae, and is not believed to appear in actively dividing conidiogenous areas. However, the term "secondary septum" does appear in the literature in this context.)

The second possibility is regrowth by branching. The patterns of conidiogenesis are identical, with or without intermittent proliferation of conidiiferous cells. If conidiogenous cycles are consecutive we have a "conidiogenous rachid", analogous to the annellide as long as it consists of a single cell (the transverse lines separating the

sequence of geniculations being mere outer scars, and not septa). If they are septa, then they could result from alternate conidiogenous cycles (with intermittent proliferations of conidiiferous cells).

"Pseudoannellations" occur in terrestrial, thick-walled hyphomycetes (*e.g. Chionomyces*), where sympodial regrowth takes place but without leaving characteristic shoulders or geniculations. The secessional scars are visible, however, on the sides of the monopodium. This phenomenon would pass unnoticed in numerous thin-walled hyphomycetes with unrecognizable scars. A "monopodium" may therefore be the result of two different types of regrowth, elongation or branching. Polyphialides are an example of a combination of mono- and sympodial regrowth processes, with secession ending successive monopodial replications only. They could also be interpreted as the result of cellular branching, depending on the sequence of appearance of the conidiogenous loci.

The complex structures known as "apical-growth phialides", annellides and sympodulae may themselves proliferate appearing in monopodial or sympodial series.

All patterns seen above, which may often appear unrelated, vary only in the relative position of secessional septa, and on whether regrowth is by elongation or by branching. Furthermore there are frequent intergradations between mono- and sympodial regrowth, which prove their low taxonomic value at the genus level or above. The fundamental similarity between apical-growth phialides and annellides was already pointed out by Minter and co-workers.

Conidia can also form by ring elongation (a term adapted from Minter and coworkers' ring wall building (*e.g.* meristem arthroconidia of *Oidium*, etc. . .)). Apical elongation presumably forms the initial element, and then is replaced by ring elongation. This type of axial regrowth can be detected by the lifting of the portion of hypha in front. When the elongating cell reaches a critical size it will undergo septation (primary). If repeated, we are dealing with a single, continuous process of regrowth from a specialized site on the conidiogenous cell. Secession may take place at a later stage, progressively or in waves, and preceded or not by secondary septation and secession of primary proconidia. These may therefore appear in continuous series above the conidiogenous cell. The ultrastructure of *Chalara* shows the deposition and separation of new internal wall layers onto the outer (lateral) wall; i.e. more than one mechanism may be involved in ring elongation. Intercalary growth has also been reported by Hughes (1953) at the base of synnemata of *Endocalyx thwaitsii*, and by Chesters (1968) in plectenchyma at the base of ascostromata. It is also the way by which higher plants and filamentous and other algae may grow. Here the process involves two steps: active cell replication in the meristem proper, followed by enlargement *above* it. In blastic conidiogenesis, though, division and elongation occur concomitantly. The term "meristematic" is thus misleading, giving the impression that the conidiogenous cell is intercalary.

When proconidia are formed after the fertile hypha stops elongating we have *thallic* conidiogenesis. Primary septation is therefore absent, but secondary septation may precede conidiogenesis, followed by lateral wall fission of alternate cells or by fission of consecutive septa.

3.3 *The proconidium*

Integrated conidiogenesis of simple conidia (also seen in species bearing compound

conidia with an integrated axis) is known to occur among hypho- and coelomycetes, and is common among the Ingoldian anamorphs. The conidiogenous hypha shows determinate *elongation*, probably not subject to tropisms, and with a characteristic orientation, (*e.g.* straight, helical, circinate, etc . . .). Primary, progressive, or, as said above, may multiple *septations* take place. This process has not been followed in detail with the high-resolution microscopy now available. As the process involves elongation, this type of conidiogenesis can be called blastic, in Kendrick's (1971) sense. After *secession*, conidiogenesis is resumed by *regrowth* identical to that in ameroconidia. Subsequent conidiogenous cycles, however, are in most cases easier to interpret, because secessional scars are visible (*i.e.* conidiogenous cells can be defined).

Branching of proconidia, (an autonomous process), either cellular or septation-dependent, gives rise to compound conidia. A detailed analysis of conidial branching patterns is beyond the purpose of this essay, but a few representative examples will be discussed. Branching at the apex produces conidia with dichotomous series (*e.g. Dwayaangam*, figure 7) or coronate conidia (*e.g. Heliscus*, figure 8). If it occurs laterally it can be opposite (*e.g. Alatospora*, figure 11) or verticillate (whorled) (*e.g. Dendrospora*, figure 12). If from the base, branching can be variously radiate, (*e.g. Lemonniera*, figure 15). In all cases these secondary elements may arise synchronously or sequentially, (again, depending probably on nuclear number). However, by far the most common pattern of conidial branching is monopodial, with branches appearing progressively in an alternate arrangement, and probably dependent on septation (*e.g. Tricladium*, figure 9). Retrogressive lateral branching is known to occur in *Taeniospora gracilis* (anam. *Leptosporomyces*, a basidiomycete, figure 19). Apical dominance of the axis may be lost, and a sympodium may be formed (*e.g. Sympodiocladium*, figure 18) Branches may undergo further *proliferation*, resulting in decompound forms such as *Varicosporium* and *Dendrosporomyces* (figure 20 and 13). Even further combinations of the above add to the structural variety displayed by compound conidia. Conidial branching structure is an important character in the artificial classification of hyphomycetes, although at the moment subjugated to the sequential patterns of secession of the sporing organ.

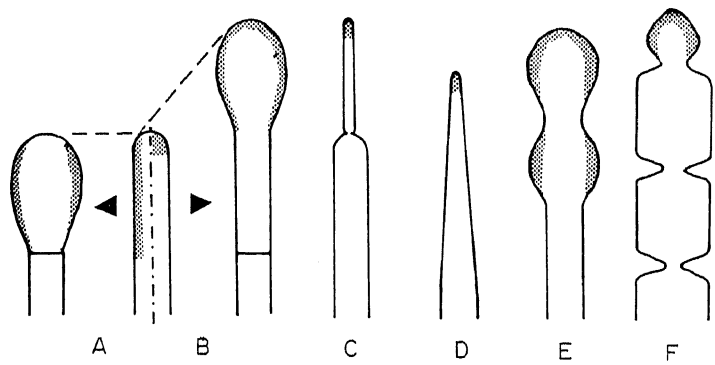
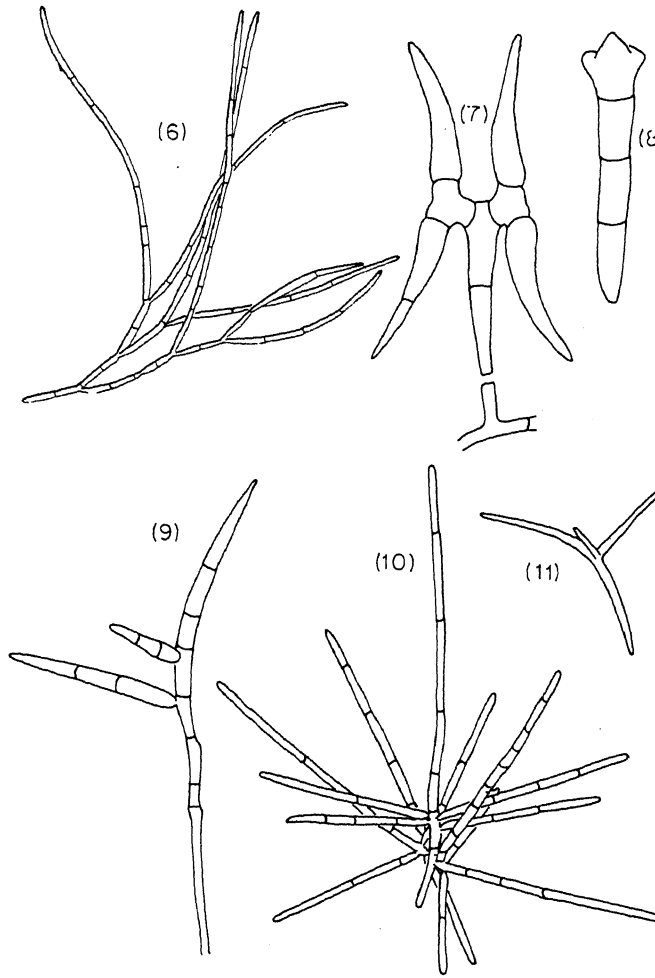
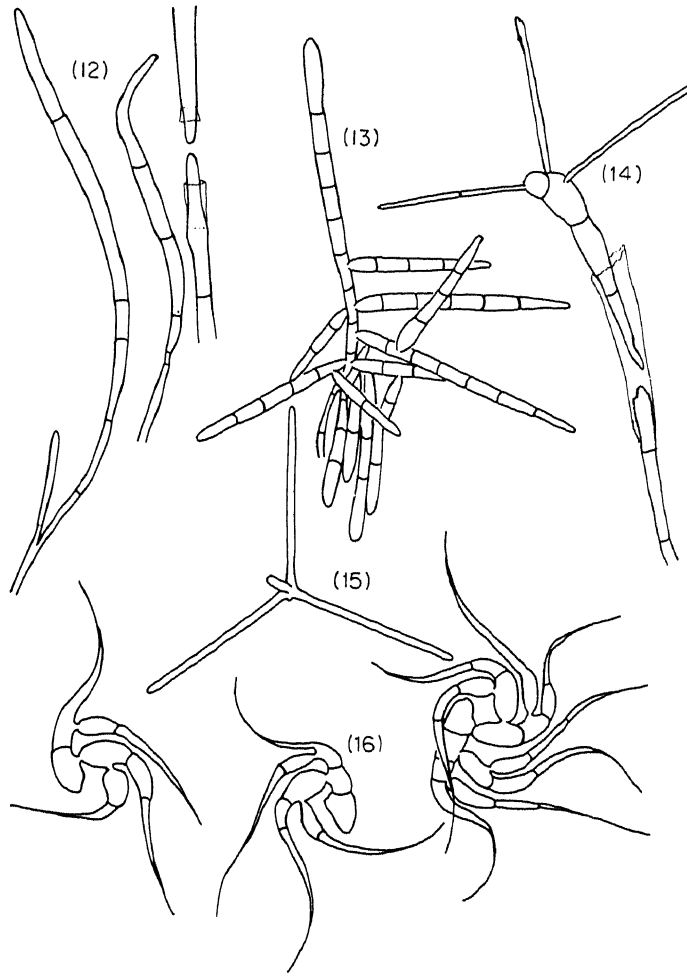


Figure 5. Diagrams of various types of transformation. **A.** Transformation by widening, without elongation. **B–F.** transformation with elongation. **B.** widening. **C–D.** Attenuation. **C.** sudden attenuation resulting in a filiform element. **D.** progressive attenuation. **E–F.** Alternation of widening and attenuation. **E.** as seen in *Monilia*, where widening may be intercalary. **F.** as seen in *Speiropsis*, where both processes occur apically (shaded areas are sites of surface increase).



Figures 6–11. **6.** *Varicosporium delicatum.* **7.** *Dwayaangam cornuta.* **8.** *Heliscus lugdunensis.* **9.** *Tricladium splendens.* **10.** *Dendrospora tenella.* **11.** *Alatospora acuminata.*

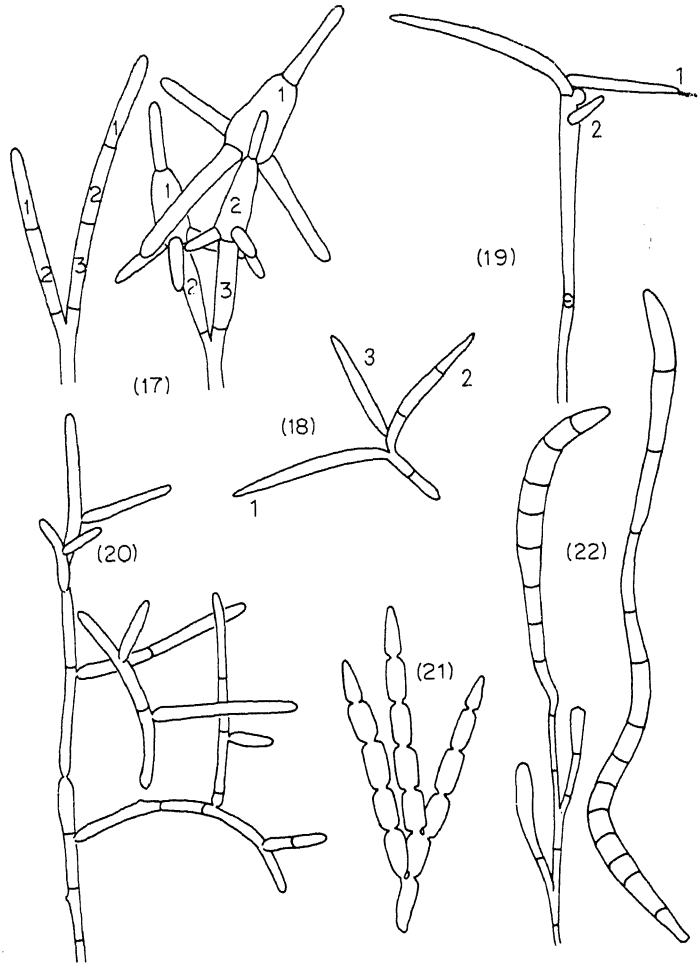
Actinospora megalospora (figure 17) is an unusual case probably combining thallic and blastic conidiogenesis. The semimacronematous conidiophores elongate apically, then forking once or twice. One or more, presumably primary, septa are laid down in each branch. Elongation then stops, and is followed immediately by the widening of the apical cell to produce a globose proconidium. Until here development is thallic. But several branches will soon appear synchronously, which will radiate by apical elongation and septate at least once, at the base (*i.e.* blastic conidiogenesis). (Minter and co-workers' "conidial maturation", as it involves only diffuse wall building, would only result in the formation of the primary globose element). After secession of the apical proconidium, the subtending cell(s) on the conidiophore branches are retrogressively transformed into further primordia in an identical fashion. There is a striking synchrony between the branches of any one conidiophore of *Actinospora*, probably related to the ease with which protoplasmic material migrates intercellularly, as seen in



Figures 12–16. **12.** *Anguillospora longissima.* **13.** *Dendrosporomyces prolifer.* **14.** *Culicidospora aquatica.* **15.** *Lemonniera aquatica.* **16.** *Gyoerffyella rotula.*

flow chamber studies (Descals *et al* 1976). Sporulation, if one ignores the formation of conidial branches, is identical to that in *Staheliella nodosa* (Cole and Samson 1980).

3.3a *Transformation*: Transformation or shape "differentiation" (Marchant 1979), may affect only cells, but also whole elements, or even organs, through integrative mechanisms which predetermine their shape. Widening may take place, as with hyphae, over the whole of the proconidial surface, or it may be limited to the apex. It can increase progressively, giving rise to clavate shapes (often the case in early conidial ontogeny); or more abruptly, the element then returning to a constant rate of transformation (*e.g.* development of conidial branches in *Varicosporium elodeae*, (figure 20), or of conidial elements in *Speiropsis* (figure 21). Attenuation, (= a reduction in the rate of transformation), however, occurs only at the elongating apex (Kendrick 1971; Cole and Samson 1980). In *Anguillospora crassa* (figure 22), the conidiogenous



Figures 17–22. **17.** *Actinospora megalospora.* **18.** *Sympodiocladium frondosum.* **19.** *Taeniospora gracilis.* **20.** *Varicosporium elodeae.* **21.** *Speiropsis pedatospora.* **22.** *Anguillospora crassa.* (figures 6 to 22 have not been done to scale).

hypha elongates and broadens gradually up to about halfway its final length; it appears until now as a clavate, integrated element. The final fusoid shape then develops by progressive attenuation, although the whole element continues broadening, probably until well after secession. In *Speiropsis* (figure 21) or *Isthmolongispora*, proconidial elements are formed by repeated alternations of widening and attenuation. That most of the transformation takes place apically is proven by the smaller size of the terminal element (the term "cell" is not used, because there may be no septa). The isthmi, therefore, do not seem to form by intercalary constriction, as could happen in structures with unset walls.

Monilia is another case of alternating widening and attenuation, except that here the process is not as well ordered, and it is followed by septation at the constrictions, and eventual secession of the individual elements.

Filiform elements, mostly referred to as appendages, are frequent in compound conidia (e.g. *Culicidospora*, (figure 14), *Clavariopsis*, *Clavatospora*, etc . . .). They elongate apically and septa may sometimes be seen along their length. Apical attenuation is retained during the entire elongation of the element, resulting in the characteristic thin, cylindrical, straight or curved structure. In other cases, attenuation may also be progressive, as seen in integrated filiform elements (e.g. *Gyoerffyella*, figure 16). Filiform elements must be distinguished from the acellular mucilaginous or setulate appendages (Sutton 1980), so common on coelomycete conidia, and also on marine ascospores, but infrequent among hyphomycetes.

3.3b *Secession*: Processes of *secession* have been amply described by others, and only a few pertinent points will be emphasised. As seen in figure 1, secession is not restricted to the base of the conidium. We already described it as a possibly random process in hyphae. If we submerge colony pieces of *Dendrospora tenella* (figure 10), an aquatic hyphomycete with highly branched conidia, in a Petri dish with water and incubate it undisturbed, not only will whole conidia eventually separate, but also parts of these (part-conidia). These are recognizable as conidial branches because of their constricted scars, instead of the broad ones found at the base of the conidia. In *Gyoerffyella rotula* (figure 16) on the other hand, and possibly in *V. elodeae*, the proliferous conidia also break up into parts, although here these are much more similar to their parent structures, and have been referred to as *secondary conidia* (Marvanová 1975).

3.4 *The free conidium*

Conidiogenesis may proceed after secession. A marked *elongation* of conidial elements at their pole(s) is frequently observed among Ingoldian hyphomycetes, but must not be taken for germination, as the growing portion of the element remains transformed. This process may be common, as it has also been noticed in *Pseudocercospora trichophila* by Deighton (1976). Compound conidia may also continue *branch* proliferation, and if their new elements are caducous, we again have a case of secondary conidiogenesis (as seen on proconidia). In *Anguillospora crassa* (figure 22) it is believed that the bifusoid propagules appearing on water in Petri dishes after prolonged submersion are the result of apical proliferation. Also a common process in detached aquatic conidia is *secondary septation* during ageing, and in some cases, the eventual breaking up of conidia into individual cells, which round up. Whether this takes place in nature is unknown. After some diffuse growth ("spherical growth" in ameroconidia, Smith and Berry 1974) conidia quickly revert to hyphal growth (= germination), which marks the end of conidiogenesis.

3.5 *The conidiophore*

Conidiophores have been called fertile hyphae, and as such, should display similar ontogenic processes.

Elongation of conidiophores is a determinate process, mostly taking place *apically*. In a few cases, however, this is soon replaced by *ring elongation*, as seen in basauxic conidiophores. This has a striking parallel in sporangiophore elongation of *Phycomyces*. Conidiophore *branching* is also autonomous, giving rise to characteristic patterns, such as verticillate, penicillate, basitonous, etc . . . It is normally progressive, but may also be retrogressive (*e.g. Graphium ulmi*, de Hoog 1974). Branching before

sporulation is distinguished from that after secession of the first crop of conidia, (= regrowth). An interesting case of apical branching is that of *Aspergillus*, where the multinucleate conidiophore apex widens (the so-called "vesicle") and undergoes synchronous, multiple branching (= synchronous cell divisions) to produce either metulae or phialides, which will eventually lay down primary septa, and continue producing further metulae, phialides or conidia, depending on the species. *Secession* of conidiophore branches is also possible, as seen in ramoconidia, or in whole branching systems such as those of *Sporidesmium arengae* (Hughes 1978).

4. Conclusions

For anamorph taxonomy to progress as a science, a universally accepted language (= descriptive terminology) is imperative. Unfortunately this is not yet available. However, a few suggestions in that direction could be proposed. The criteria used, for example, should be clearly recognised. Among those strictly morphological we could use the following independently: *karyological* (referring to nuclei), *cytological* (with the cell as the unit), and *structural* (pertaining to elements and organs). These are based on the fact that growth and form are genetically controlled at independent levels: cellular and intercellular. Terms must be compared at the same level of observation, *i.e.* we must distinguish between ultrastructural and microstructural terms, (observed respectively with the electron and light microscopes). The former refer to mechanisms (such as wall synthesis and lamination) by which processes take place; and, as seen above, more than one mechanism may produce the same effect (*e.g.* cell elongation by apical or ring wall building). Ontogenic criteria should be kept separate from those referring to the mature structure. For example, karyological criteria could compare nuclear behaviour at cell division, or just nuclear numbers and arrangement; cytological criteria should apply to numbers and arrangement of cells (or septa) separately from the ways by which these are attained, that is, the methods and sequence of cell divisions; structural criteria, likewise, should describe processes of transformation, secession, etc . . . , independently of their outcome, *i.e.* the final shape of the element; or they may apply to types of sequence of branching as distinct from the mere arrangement of elements into organs.

Because morphology deals with the study of form, it does not help to refer to unrecognizable entities, (*e.g.* proconidia in integrated conidiogenesis). Terms referring to different processes should not overlap in their definitions; that is, any single permanent change of form must be explained with a single morphological process. For example, it does not seem necessary to say that a part of a conidiophore widens by joint processes of "maturation and proliferation", (in Minter and co-workers' sense). As already proposed by these authors, developmental terms must be used only when the processes they refer to are taking place. No hierarchy should be established in the use of criteria unless there is genetic evidence for it. This means that any system proposed at this stage for anamorphs can only be experimental.

The whole fungus is formed by the same developmental principles at the cytological and structural levels. Therefore the anamorph should be studied as a whole, *i.e.* mycelium, fertile organs and propagules. For example, patterns of septation (progressive and multiple) and branching (depending or not on secession, or on septation) seen in hyphal morphogenesis, could, in the future, help define more clearly those observed in conidiogenesis.

With the above suggestions in mind, several guidelines can be proposed, pertaining to (a) the mature structure and (b) developmental processes.

(a) Mature structures (cells and hyphae, or elements, and organs) need to be described only with the seven structural criteria proposed above. This standardization should lead to a clearer observation and understanding of the patterns involved, and it would also avoid unnecessary repetition or omission of characters.

(b) The following ontogenic characters are only preliminary, and no doubt will be altered in the near future, but they could be considered as a continuation of the dialogue already initiated by Minter and co-workers, though extended to more complex patterns of conidiogenesis involving branched and integrated structures. The first character is *growth* (morphological) of elements and organs, which can then be broken down into *elongation, branching*, and *transformation*, and various combinations of these. If cells are used as the recognizable units for description, one would refer to processes of *cell division*, (*i.e.* septation) or *cell lysis* affecting septal or outer walls (as in dolipore dissolution, and outer wall fission) or affecting protoplasm (*e.g.* cell autolysis prior to rhexolysis, etc . . .). Secondly there is *secession* in its various forms, which is in a way a specialization of lytic processes. Thirdly, one must consider *maturation* processes, affecting either walls or protoplasm. All the above can also be combined to explain observed patterns of *integrated morphogenesis*. Several examples have been given.

Morphological processes, however, will be taxonomically stable only if they can meet two requirements: they must eventually be correlated with genetic processes, which ultimately control growth and form; and they must have an adaptive value in the various events leading to the survival of the species (*e.g.* dispersal, colonization, recombination, etc.)

Referring specifically to the aquatic hyphomycetes, Ingold (1975) suggested that they may have reverted to the aquatic habitat from a terrestrial ancestry. From the discussion above, however, one could speculate that there could be an evolutionary continuum starting with the undifferentiated branched hyphal monopodium, with random, sporadic fragmentation and regrowth. Fragmentation could then have gradually localized itself in convenient sites, depending on selection pressures. In aquatic habitats an intermediate situation resulting in hyphoid, branched propagules would be best adapted to anchorage in a moving medium, (Webster and Davey 1984), or to displacement on surface films (Bandoni 1974) (as in soils and leaf litter, which are often considered to have an aquatic component). In increasingly terrestrial habitats, secession could have displaced itself to the uppermost portion of the hypha, culminating in the separation of only the distal cell from the thallus (= the ameroconidium, ideal for aerial dispersal). Whether evolution has operated in this way, or in that proposed by Ingold, or even in more than one direction, is, however, still a matter for conjecture.

Acknowledgements

Thanks are due to Prof. J Webster, Mr N Money, Prof. G Hennebert and Dr D Minter for having read the manuscript or held useful discussions on terminology. The author wishes to thank Prof. C V Subramanian for his kindness and encouragement throughout their years of friendship.

References

Bandoni R J 1974 Mycological observations on the aqueous films covering decaying leaves and other litter; *Trans. Mycol. Soc. Jpn.* **15** 309–315

Barron G L 1972 *The genera of hyphomycetes from soil* (New York: Krieger Pub. Co.)

Beverwijk A L van 1951 Zalewski's *Clathrosphaera spirifera; Trans. Br. Mycol. Soc.* **34** 280–290

Chesters C G C 1968 Morphology as a taxonomic criterion. In *The Fungi; an advanced treatise.* (eds) G C Ainsworth and A S Sussman (London: Academic Press) vol. III, 517–542

Clutterbuck A J 1970 Synchronous nuclear division and septation in *Aspergillus nidulans; J. Gen. Microbiol.* **60** 133–135

Cole G C and Samson R A 1979 *Patterns of development in conidial fungi* (London: Pitman).

Deighton F C 1976 Studies on *Cercospora* and allied genera, VI; *Commonw. Mycol. Inst. Mycol. Pap.* **140** 1–168

Descals E, Nawawi A and Webster J 1976 Developmental studies in *Actinospora* and three similar aquatic hyphomycetes; *Trans. Br. Mycol. Soc.* **67** 207–222

Ellis M B 1971 *Dematiaceous Hyphomycetes* (Kew: Commonw. Mycol. Inst.)

Fiddy C and Trinci A P J 1976 Mitosis, septation, branching and the duplication cycle in *Aspergillus nidulans; J. Gen. Microbiol.* **97** 169–184

Griffin D H 1981 *Fungal Physiology* (New York: John Wiley)

Hammill T M 1981 Conidiogenesis. In *The Fungal Spore: morphogenetic controls* (eds) G Turian and H R Hohl (New York: Academic Press) 151–171

Hoog G S 1974 The genera *Blastobotrys, Sporothrix, Calcarisporium* and *Calcarisporiella* gen. nov. Baarn, Centraalbureau voor Schimmelcultures.

Hughes S J 1951 Studies on microfungi VII; *Commonw. Mycol. Inst. Mycol. Pap.* **41** 1–17

Hughes S J 1953 Fungi from the Gold Coast. II; *Commonw. Mycol. Inst. Mycol. Pap.* **50** 1–104

Hughes S J 1978 New Zealand Fungi, 25. Miscellaneous species; *N. Z. J. Bot.* **16** 311–370

Ingold C T 1975 Convergent evolution in aquatic fungi: the tetraradiate spore; *Biol. J. Linn. Soc.* 7 1–25

Kendrick B 1971 Conidium shape. In *Taxonomy of Fungi Imperfecti* (ed.) B Kendrick (Toronto: Univ. of Toronto Press) 301–304

Kendrick B and Nag Raj T R 1979 Morphological terms in *Fungi Imperfecti.* In *The Whole Fungus; the sexual-asexual synthesis* (ed.) B Kendrick (Ottawa: Nat. Mus. of Nat. Sci. and Kananaskis Foundation) 43–61

Madelin M F 1981 Ultrastructural morphogenesis in higher fungi; discussant's introduction. In *The fungal spore; morphogenetic controls* (eds) G Turian and H R Hohl (New York: Academic Press) 95–106

Marchant R 1979 Wall growth during spore differentiation and germination. In *Fungal walls and hyphal growth* (eds) J H Burnett and A P J Trinci (Cambridge: C.U.P.) 115–148

Marvanová L 1975 Concerning *Gyoerffyella* Kol; *Trans. Br. Mycol. Soc.* **65** 555–565

MacLaughlin D J 1977 Basidiospore initiation and early development in *Coprinus cinereus; Am. J. Bot.* **64** 1–16

Minter D W, Sutton B C and Brady B L 1983 What are phialides anyway?; *Trans. Br. Mycol. Soc.* **81** 109–120

Prosser J I 1979 Mathematical modelling of mycelial growth. In *Fungal walls and hyphal growth.* (eds) J H Burnett and A P J Trinci (Cambridge: C.U.P.) 359–384

Prosser J I and Trinci A P J 1979 A model of hyphal growth and branching; *J. Gen. Microbiol.* **111** 153–164

Robertson N F 1958 Observations on the effect of water on the hyphal apices of *Fusarium oxysporum; Ann. Bot. London* **22** 159–173

Robertson N F 1959 Experimental control of hyphal branching and branch form in hyphomycetous fungi; *J. Linn. Soc. Bot.* **56** 207–211

Smith J E 1978 Asexual sporulation in filamentous fungi. In *The filamentous fungi* (eds) J E Smith and D R Berry (London: E. Arnold) vol. 3 214–239

Smith J E and Berry D R 1974 *An introduction to biochemistry of fungal development* (London: Academic Press)

Stearn W T 1973 *Botanical Latin* (Newton Abbot: David and Charles)

Sutton B C 1980 *The Coelomycetes* (Kew: Commonw. Mycol. Inst.)

Trinci A P J 1978 The duplication cycle and vegetative development in moulds. In *The filamentous fungi* (eds) J E Smith and D R Berry (London: E. Arnold) vol 3 132–163

Trinci A P J 1984 Regulation of hyphal branching and hyphal orientation. In *The ecology and physiology of the fungal mycelium* (eds) D H Jennings and A D M Rayner (Cambridge: C.U.P.) 23–52

Webster J and Davey R A 1984 Sigmoid conidial shape in aquatic fungi; *Trans. Br. Mycol. Soc.* **83** 43–52

Webster J, Nawawi A and Descals E 1975 *Spore development in aquatic hyphomycetes* 16 mm ciné film, Univ. of Exeter, Great Britain.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 229–244.
© Printed in India.

Notes on some deuteromycete genera with cheiroid or digitate brown conidia

B C SUTTON

Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF, UK

Abstract. The generic names *Chelisporium* and *Cheiroconium* are reduced to synonymy with *Sirothecium* and three species are accepted. *Cheiromycella* is briefly reviewed and *Cheiromyces* is maintained with a single species. Its typification is discussed. *Dictyosporium* is briefly discussed and a new species, *D. australiense*, is introduced. The name *D. subramanianii* sp. nov. is proposed for the invalidly published *D. intermedium* and *D. circinatum* is transferred to *Helicorhoidion*. *Digitodesmium* is doubtfully kept distinct from *Cheiromyces*.

Keywords. *Chelisporium; Cheiroconium; Sirothecium; Dictyosporium; Helicorhoidion; Digitodesmium.*

1. Introduction

Three separate events led to the preparation of this paper. The discovery of an isotype of *Chelisporium hysterioides*, described from *Eucalyptus*, in the Petrak herbarium in herb. W prompted a comparison with similar fungi because this genus had oscillated between the hyphomycetes and coelomycetes in various compilations and schemes of classification over the genus since its description in 1910. Secondly Dr J L Alcorn and I made a collection on *Eucalyptus* in 1981 in Australia which did not fit with any of the well-known cheiroid species of hyphomycete and it therefore became necessary to examine several similar genera to determine its affinities. In addition to *Chelisporium* this complex of fungi includes the genera *Cheiroconium, Cheiromycella, Cheiromyces, Dictyosporium, Digitodesmium, Sirothecium* and their various synonyms. Thirdly I was pleased and honoured to accept the invitation to submit a contribution to celebrate the 60th birthday of Prof. C V Subramanian. It seemed most appropriate that this work be prepared for such an event, considering his significant contribution to hyphomycete taxonomy for many years.

2. *Chelisporium*

The genus *Chelisporium* was introduced by Speggazini (1910) with a single species *C. hysterioides* Speg., described from decorticated wood of *Eucalyptus globulus* collected in La Plata, Argentina. It was referred to as a dematiaceous *Dicranidion* with erumpent conidiomata and cheliform (claw-like) conidia consisting of a basal single cell surmounted by parallel often plurilocular arms. The species was well-illustrated although no details of conidiogenesis or conidiophores were given, a not unusual occurrence for accounts of that time. Saccardo (1911) and Saccardo and Trotter (1913) assigned *Chelisporium* to the hyphomycetes in the Tuberculariaceae and Höhnel (1923) included it in the Tuberculariеen, Pachytuberculariеen, Dematieae. It is listed as a coelomycete in Hawksworth *et al* (1983) without comment but included in the

hyphomycetes by Carmichael *et al* (1980) with a question mark. There have been no additions to the genus and no detailed subsequent accounts of the single species have appeared.

The isotype of *C. hysterioides* found in herb. W has enabled a more complete description of the species to be made (figures 1 A–C).

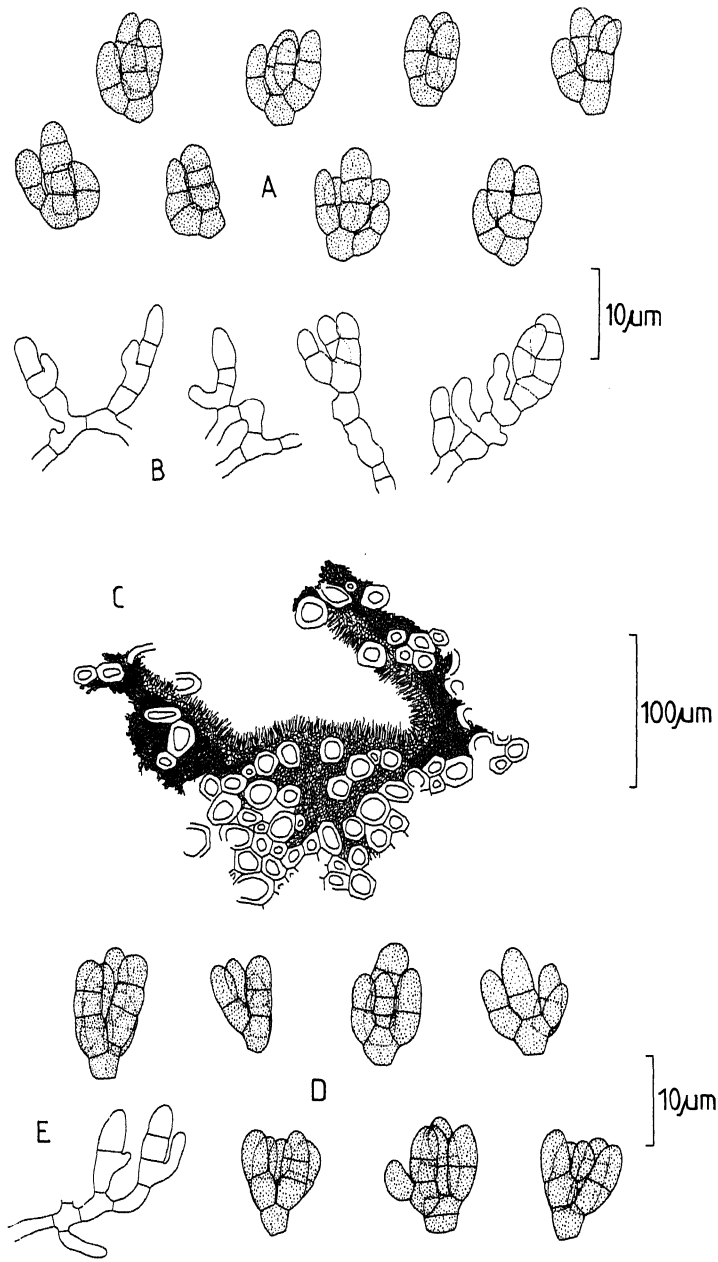


Figure 1. *Sirothecium tinctum.* **A–C.** isotype of *Chelisporium hysterioides.* **A.** conidia; **B.** conidiophores and developing conidia; **C.** vertical section of a conidioma. **D–E.** isotype of *Cheiroconium beaumontii.* **D.** conidia; **E.** conidiophore with young conidia.

Mycelium is immersed, branched, septate, hyaline to pale brown, extensive, 2–3 μm wide. *Conidiomata* are eustromatic, immersed to semi-immersed, ellipsoid to navicular or hysteriiform, dark brown to black, unilocular, 550 μm long × 270 μm wide × 180 μm high; upper and lateral walls several cells thick, composed of dark brown textura angularis on the outside, lined by hyaline to pale brown similar tissue, at the base only composed of the paler cells. *Dehiscence* by a longitudinal split in the upper wall, eventually with the overlying tissue breaking down to leave an open conidioma. *Conidiophores* hyaline, repeatedly and irregularly branched, septate, 1·5–2·5 μm wide, vertically orientated, formed from the inner cells of the conidiomatal wall. *Conidiogenous cells* integrated, apical, lateral or comprising the conidiophore, hyaline, smooth, producing a single conidium or occasionally a second one from an additional conidiogenous locus. *Conidia* holoblastic, overall measuring 10·5–13 × 6–9 μm, pale to medium brown, smooth, eguttulate, closely branched, consisting of a truncate basal cell 3·5–4 μm wide on which 2–4 vertical slightly divergent arms are inserted in different planes. Arms 0–2 euseptate, slightly constricted at septa, occasionally branched, of variable length in any single conidium, 5–9·5 μm long × 2·5–3·5 μm wide, apical cell obtuse.

These features agree well with those of the coelomycete genus *Sirothecium* Karst. (Sutton 1978; Morgan-Jones *et al* 1972) so the generic and specific names *Chelisporium* and *C. hysterioides* are reduced to synonymy with *Sirothecium* and *S. tinctum* respectively.

Material examined: on *Eucalyptus globulus*, La Plata, Argentina, C. Spegazzini, 23 Oct. 1906, isotype of *Chelisporium hysterioides* Speg., IMI 286915 ex W.

3. *Cheiroconium*

The reasons which led Höhnel (1910) to introduce the generic name *Cheiroconium*, based on *C. beaumontii* Höhnel (in error cited as *C. beaumontii* (Berk. & Curt.) Höhnel) are explained in the discussion on the genus *Cheiromyces* Berk. which follows. Only the single species has been placed in the genus. Damon (1950), because of his misconceptions over lectotypification of *Cheiromyces*, erroneously regarded *Cheiroconium* as a synonym of *Cheiromyces*. Later, Hughes (1958) placed *Cheiroconium* in synonymy with *Sirothecium* Karst., a coelomycete genus. Morgan-Jones *et al* (1972) and Sutton (1978) have given modern illustrated accounts of the genus. The synonymy is confirmed by examination of the Berkeley specimen no. 5087 (named as *Cheiromyces stellatus* by Berkeley, 1875) where conidiomatal structure, conidiophore morphology, conidiogenesis and conidial size and morphology are almost identical (figure 1, D–E). The conidia of *Cheiroconium beaumontii* are slightly more complex than in *Chelisporium hysterioides* but are very similar in size (11–14·5 × 7–10 μm for *C. beaumontii*, 10·5–13 × 6–9 μm for *C. hysterioides*). Thus these names are reduced to synonymy with *S. tinctum*.

Material examined: on decorticated wood, Alabama, USA, leg. Beaumont, no. 5087, isotype of *Cheiroconium beaumontii* Höhn., IMI 287049 ex K.

4. *Cheiromycella*

In the reassessment of *Cheiromyces* made by Höhnel (1910) the new generic name *Cheiromycella* was introduced for a species he had described earlier as *Cheiromyces*

speiroides Höhnel (1903). Mason and Hughes in Rimington (1953) added *C. gyrosa* to the genus but later Hughes (1958) showed that *Dicoccum microscopicum* Karst. provided an earlier name for this reasonably common mainly coniferous species. He proposed a full synonymy which included *C. speiroides*. On the basis of the original figures and description of *Coniothecium chomatosporum* Corda (1837), Boerema *et al* (1973) proposed the new combination *Cheiromycella chomatospora* for this species, but confirmation is required by examining Corda's type, for his illustration is not convincingly that of the species recognisable as *C. microscopica*.

The type species of *Cheiromycella* has been fully illustrated and described by Ellis (1971) and is represented in herb. IMI by 30 collections on various coniferous substrates and undetermined wood. It is quite distinct from *Cheiromyces* because it has punctiform sporodochial rather than effuse conidiomata, branched septate conidiophores in which more than one conidiogenous locus is often produced by each conidiogenous cell and brown euseptate conidia in which usually no more than two arms are produced by the basal conidial cell. It differs from *Sirothecium* which has hysteriiform eustromatic immersed conidiomata but otherwise the genera are similar. Conidiogenesis is holoblastic, but in *Sirothecium* the involved hyphae are hyaline rather than pale brown. The conidia in *Cheiromycella* are usually very simple, only occasionally developing more than the standard two conidial arms whereas in *Sirothecium* up to four arms are produced. An illustration from IMI 89589 is provided here to demonstrate the differences between the three genera (figure 2).

Material examined (selected): on *Larix* sp., Buttercombe, Yorks., UK, C. Booth, 18 Sept. 1961, IMI 89589.

Species placed in *Cheiromycella*.

1) *C. annulata* Matsushima, *Icones Fung. a Matsushima Lect.* 26 (1975).
This species was considered to be the same as *Helminthosporium subopacum* Cke & Ell. which provided the basionym for *Eversia subopaca* (Cke & Ell.) Crane and Schoknecht the type species of *Eversia* Crane and Schoknecht by Schoknecht and Crane (1977).
2) *C. chomatosporum* (Corda) Boerema *et al*; *Persoonia* **7** 132 (1973)
Coniothecium chomatosporum Corda, *Icon. huc. cogn.* **1** 2 (1837)
The suggestion that this provided the earliest available epithet for the type species of *Cheiromycella* needs verification by examination of the original material.
3) *C. gyrosa* (Cke & Massee) Mason & Hughes in Rimington, *Nat. Hist. Scarborough Distr.* **1** 156 (1953)
Torula gyrosa Cke & Massee apud Cooke, *Grevillea* **16** 10 (1887).
A later name for *C. microscopica*, fide Hughes (1958).
4) *C. jafarii* Rehana Begum (as '*jafaria*'), *Geobios* **5** 130 (1978)
The illustrations of flattened conidia suggest *Dictyosporium* rather than *Cheiromycella* but without examining the material it is not possible to confirm the disposition in *Cheiromycella*.
5) *C. microscopica* (Karst.) Hughes, *Can. J. Bot.* **36** 747 (1958)
Dicoccum microscopicum Karst., *Medd. Soc. Fl. Fauna Fenn.* **14** 91 (1887).
Type species of the genus.
6) *C. moniliphora* Matsushima, *Icones Fung. a Matsushima Lect.* 27 (1975)
Accepted in *Cheiromycella*. A distinctive species with basically two arms to the conidia, occasionally with three arms. The apices are typically incurved, paler and sometimes swollen.

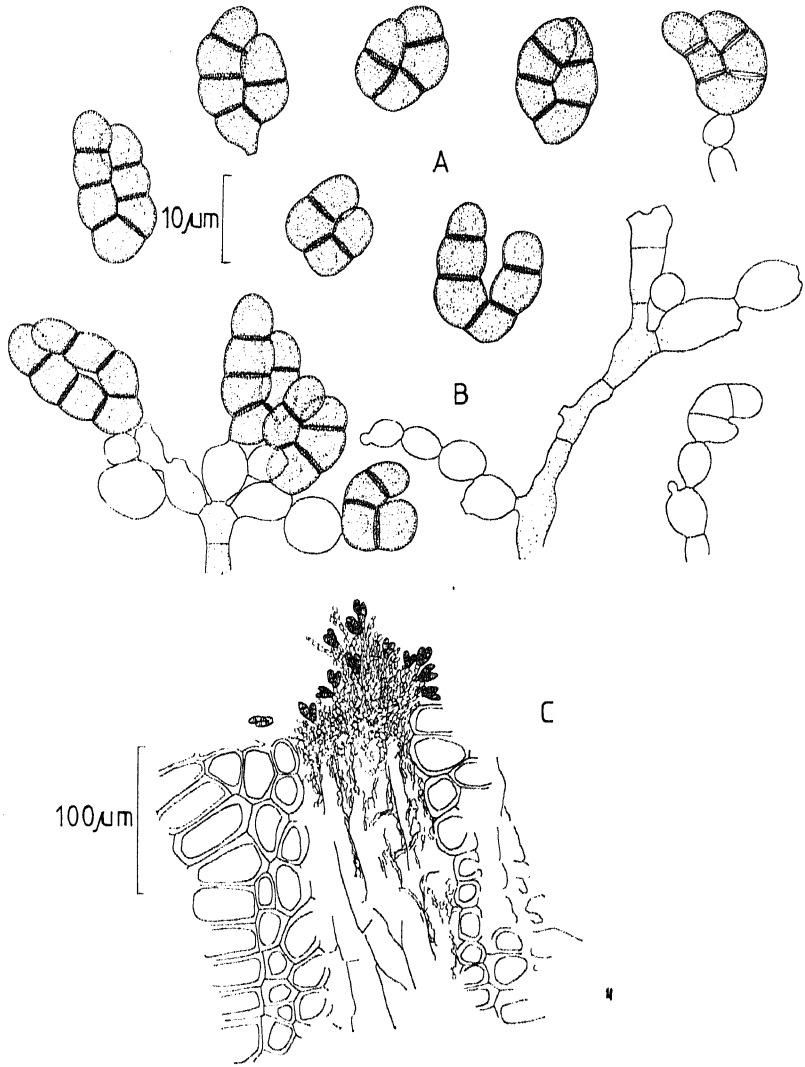


Figure 2. *Cheiromycella microscopica* (IMI 89589). **A.** conidia; **B.** conidiophores and developing conidia; **C.** vertical section of a conidioma.

7) *C. speiroides* (Höhn.) Höhn., *Sber. Akad. Wiss. Wien* **119** 664 (1910)
Cheiromyces speiroides Höhn., *Annals mycol.* **1** 408 (1903)
A later name for *C. microscopica*, fide Hughes (1958).

5. *Cheiromyces*

The history of *Cheiromyces* Berk. & Curtis has been fully documented by Martin (1944) but it has not been substantiated by full descriptions or adequate illustrations. The generic name was first used by Berkeley (1857) and although there was no detailed description, the single species, *Cheiromyces stellatus* Berk. & Curtis, was illustrated

from a specimen on *Scirpus eriophorus* sent from Pennsylvania, USA by Michener. The fungus was simply said to have a cellular base on which digitate spores are produced. No septa were shown. Later, Berkeley (1875) formally introduced the generic name, the description reading "Sporae digitatae e pulvinulo papillato orundae". The single species *C. stellatus* (spelled incorrectly as *stillatus*) was described from two specimens, the earlier one on *Scirpus eriophorus* (no. 4883) and a second "on a *Sphaeropsis*" by Beaumont from Alabama (no. 5087). The account read "Forming little specks, which consist of a cellular papillose cushion, to which are fixed the digitate spores, 0·0006 long. The divisions, which are obtuse, vary from two to five, and are sometimes waved". Martin (1944) considered the Alabama collection (no. 5087) as possibly the source of the material distributed by Curtis as *C. beaumontii* B. & C. but this name was not validly published and doubt has been expressed as to whether it is conspecific or even congeneric with the other collection from Pennsylvania (no. 4883). Although Höhnel (1910) failed to find the fungus on the type of *C. stellatus* in herb. K he deduced from the description and illustration of the species and the examination of '*C. beaumontii*' material that the two collections are neither congeneric nor conspecific. He maintained *Cheiromyces* for *C. stellatus* with the implicit lectotype as collection no. 4883, and removed '*C. beaumontii*' to a new genus, *Cheiroconium*. In addition he introduced the generic name *Cheiromycella* to accommodate a species he described earlier as *Cheiromyces speiroides* Höhnel (1903). In notes furnished by G R Bisby (filed in herb. K) for Martin's studies it is stated that "A bit of this specimen [no. 5087] from Cooke's Herb., Ellis's N A Fungi 762, and Cooke's Fungi-New Jersey, USA No. 3484 were also sent to von Höhnel, who (Fragm. 562) redescribed the fungus as *Cheiroconium beaumontii*". Thus specimen no. 5087 is designated here as the lectotype for *C. beaumontii* Höhn. which is fully described for the first time by Höhnel (1910). Unfortunately Damon (1950) in failing to examine material of *C. stellatus* (no. 4883) took-up his interpretation of *Cheiromyces* based on no. 5087 and this resulted in his reduction of *Cheiroconium* and *Cheiromycella* to synonymy with *Cheiromyces*.

The following accounts of *Cheiromyces* and its single species *C. stellatus* (figure 3) are based on no. 4883.

Cheiromyces Berk. & Curtis, *Grevillea* **3** 97 (1875)

Conidiomata separate, sporodochial, applanate, brown, consisting of textura angularis. *Conidiophores* absent. *Conidiogenous cells* discrete, brown, smooth, doliiform, formed from the upper cells of the conidiomata. *Conidia* holoblastic, pale brown, smooth, consisting of a truncate basal cell on which 3–5, straight, vertical, appressed or slightly divergent, cylindrical, distoseptate arms are formed in different planes. *Development of conidia*: holoblastic conidial ontogeny, delimination by a transverse septum, maturation by apical wall building, secession schizolytic, with no conidiogenous cell proliferation.
Type species *C. stellatus* Berk. & Curtis

Cheiromyces stellatus Berk. & Curtis, *Grevillea* **3** 97 (1875).

Mycelium sparse, immersed and branched, septate, hyaline, 2 μm wide. *Conidiomata* sporodochial, separate, discrete, superficial, applanate, circular to ellipsoid, golden brown, up to 210 μm diam; basal wall consisting of pale brown, thin-walled textura angularis 2–3 cells thick. *Conidiophores* absent. *Conidiogenous cells* discrete, pale

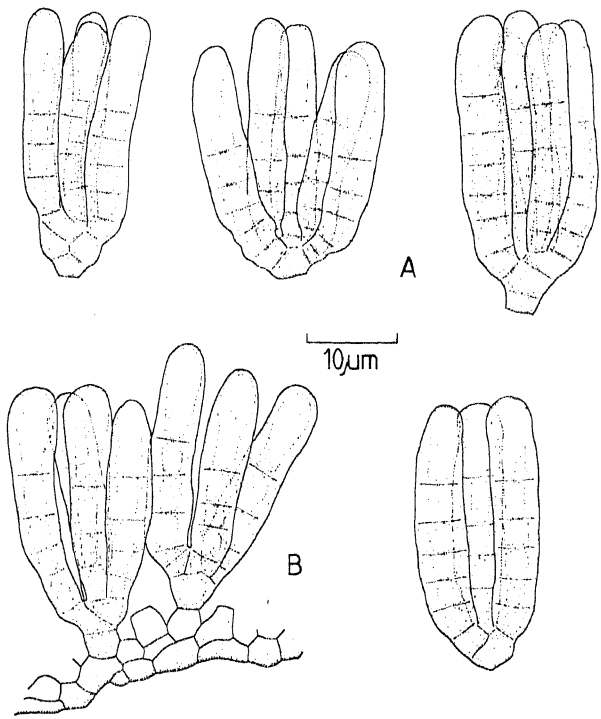


Figure 3. *Cheiromyces stellatus*, lectotype of *C. stellatus*. **A.** conidia; **B.** basal part of the conidioma with developing conidia.

brown, smooth, doliiform, 4–5 µm long × 3·5–4 µm wide, formed from the upper cells of the conidiomata. *Conidia* holoblastic, pale brown, smooth, closely branched, consisting of a truncate basal cell 4–5·5 µm wide on which 3–5 vertical appressed or slightly divergent, cylindrical arms are inserted in different planes. Arms 3–5 distoseptate with much reduced cell lumina, not constricted at the septa, unbranched, of more or less similar length, 23–30 µm long × 4–6 µm wide, apical cell longer than the rest and slightly wider.

Specimen examined: on *Scirpus eriophorum*, Pennsylvania, USA, leg. Michener, no. 4883, lectotype of *Cheiromyces stellatus* Berk. & Curtis, IMI 287050 ex K.
Species placed in *Cheiromyces*.

1) *C. acutus* Müller & Shoem., *Nova Hedwigia* 7 2 (1964)
   The pointed divergent euseptate arms of the conidia indicate that this species is incorrectly placed in *Cheiromyces* but the original collection requires examination before an alternative genus can be suggested.
2) *C. beaumontii* Berk. & Curt., nom. nud.
   A synonym of *Sirothecium tinctum* (Peck) Hughes.
3) C. *bulbophorus* Mats., *Mycol. Mem.* **3** 3 (1983)
   The euseptate conidia and dematiaceous pigment of the conidiogenous apparatus are incorrect for *Cheiromyces*. The species might be better placed in *Cheiromycella* Höhn.

4) *C. comatus* Ell. & Ev., *Proc. Acad. Phil. 1893* 171 (1893)
Höhnel (1903) first transferred this name to *Exosporium* Link and later (Höhnel, 1914) to *Cryptocoryneum* Fuckel.
5) *C. digitatus* G. W. Martin, *J. Wash. Acad. Sci.* **34** 359 (1944)
Although Damon (1950) transferred this name to *Speira* Corda, later (Damon 1952) he placed the names in synonymy with *Dictyosporium zeylanicum* Petch.
6) *C. inflatus* Mats., *Mycol. Mem.* **3** 3 (1983)
The euseptate conidia are incorrect for *Cheiromyces*. The species might be better placed in *Cheiromycella* Höhn.
7) *C. montellicus* (Sacc.) R T Moore, *Mycologia* **50** 682 (1959).
Stigmella montellica Sacc., *Michelia* **1** 90 (1877)
The dark brown euseptate conidia which are constricted at the septa are not correct for *Cheiromyces* but it is not possible from the account by Moore (1959) to suggest an alternative generic placement.
8) *C. speiroides* Höhn., *Annls mycol.* **1** 408 (1903).
Placed as a synonym of *Cheiromycella microscopica* (Karst.) Hughes by Hughes (1958).
9) *C. stellatus* Berk. & Curtis, *Grevillea* **3** 97 (1875)
Type species of the genus.
10) *C. tinctus* Peck, *Bot. Gaz.* **5** 35 (1880)
This name was transferred to *Sirothecium* as *S. tinctum* (Peck) Hughes by Hughes (1958).

6. *Dictyosporium*

The history of *Dictyosporium* Corda (1836) has been fully described by Damon (1952) who suggested a generic synonymy which included *Speira* Corda (1837) and *Cattanea* Garovaglio (1875). *Synphragmidium* Strauss (1853) was also questionably listed as a synonym but neither Hughes (1958) nor Ellis (1971) accepted this disposition and Carmichael *et al* (1980) query it as a nomen dubium. Saccardo (1886) places it as a synonym of *Speira*. Damon (1952) illustrated and described seven species and his concept of the genus was reasonably homogeneous. It formed the basis of the shorter modern account by Ellis (1971) where four species were illustrated and described. Five taxa described in the genus were excluded from it by Damon (1952). Since this time there have been several additions to *Dictyosporium*, some of questionable acceptance. As presently conceived the genus is characterized by colonies which may be compact and sporodochial or effuse, arising from a mostly immersed mycelium. Conidiophores as such are absent but the conidiogenous cells which arise direct from the hyphae and cells constituting the conidiomata are discrete and doliiform. They produce a single conidium from each conidiogenous cell. Conidia are holoblastic, solitary, brown, cheiroid, sometimes flattened in one plane, closely branched from the base, euseptate, without the arms separating.

The species collected in Australia is similar in overall morphology to *D. minor* (Sacc.) Damon but the conidia are larger, and have more septa. It has also been compared with the original accounts and modern treatments, where they exist, of other species in the genus and none so far described will satisfactorily accommodate it.

***Dictyosporium australiense* sp. nov.**

Mycelium immersum vel superficiale, ramosum, septatum, pallide brunneum, extensum, 2–2·5 μm crassum. Conidiomata sporodochialia, separata vel confluentia, superficialia, applanata, circularia vel ellipsoidea, brunnea, usque ad 100 μm diam, strato basali ex textura angulare pallide brunnea vel hyalina, parietibus tenuibus, 2–3 cellulis crassis composito. Conidiophora hyalina vel pallide brunnea, irregulariter ramosa, breve, septata, 5–17·5 × 2–2·5 μm, ex cellulis superioribus conidiomatum formata. Cellulae conidiogenae discretae vel in conidiophoris incorporatae, pallide brunneae, laeves, doliiformes vel cylindricae, 2·5 μm longae × 2·5–3 μm crassae. Conidia 36–43 × 11·5–12 μm, holoblastica, pallide brunnea, laevia, arcte appressa, cellula basale truncata 4–5·5 μm crassa, tribus brachiis verticalibus vel leniter curvatis, non complanata. Brachia 7–11 euseptata, ad septis constricta, non ramosa, 27·5–37 × 5·5–7 μm, cellulo apicale conico vel obtuso.

In lignis *Eucalypti*, Mt Glorious State Forest, Queensland, Australia, B.C. Sutton & J. L. Alcorn, 27 Aug. 1981, IMI 263318*a*, holotypus.

Mycelium immersed or superficial, branched, septate, pale brown, extensive, 2–2·3 μm wide. *Conidiomata* sporodochial, separate or confluent, superficial, applanate, circular to ellipsoid, brown, up to 100 μm diam, basal layer consisting of pale brown to hyaline, thin-walled textura angularis 2–3 cells thick. *Conidiophores* hyaline to pale brown, irregularly branched, short, septate, 5–17·5 × 2–2·5 μm, formed from

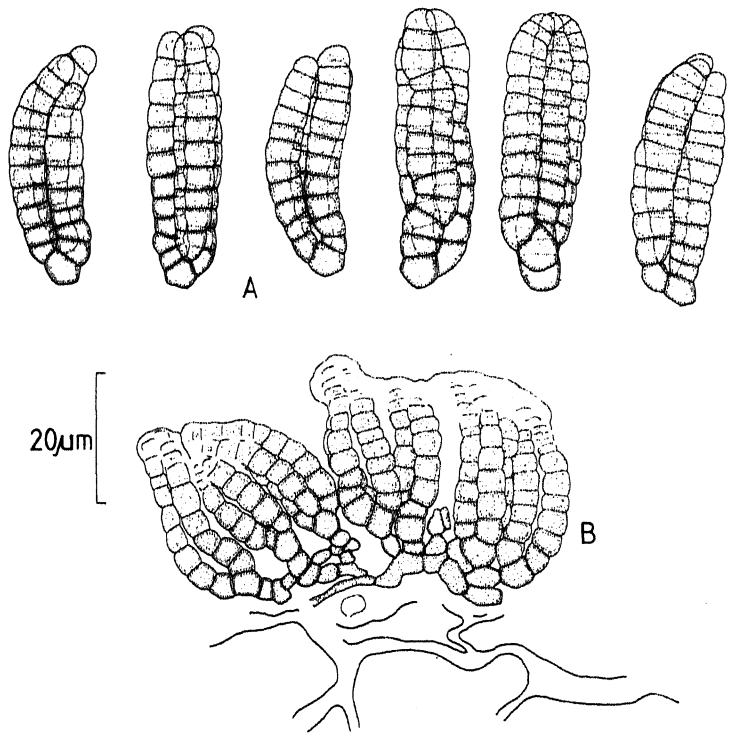


Figure 4. *Dictyosporium australiense*, holotype. **A.** conidia; **B.** vertical section of conidioma showing developing conidia.

the upper cells of the conidiomata. *Conidiogenous cells* discrete or integrated, pale brown smooth, doliiform to cylindrical, 2–5 μm long × 2·5–3 μm wide. *Conidia* 36–43 × 11·5–12 μm, holoblastic, pale brown, smooth, closely appressed, consisting of a truncate basal cell 4–5·5 μm wide on which 3 vertical, straight or slightly curved arms are inserted in different planes. Arms 7–11 euseptate, markedly constricted at the septa, unbranched, more or less of similar length, 27·5–37 × 5·5–7 μm, apical cell conic or obtuse.

Species placed in *Dictyosporium*.

1) *D. alatum* van Emden, *Acta bot. neerl.* **24** 193 (1975).
This seems to be an acceptable species, but for the hyaline appendages on the conidia.
2) *D. binatum* (Sacc.) Hughes, *Can. J. Bot.* **36** 762 (1958)
Speira binata Sacc., *Bull. Ort. Bot. Napoli* **6** 72 (1921)
3) *D. boydii* A. L. Smith & Ramsb., *Trans. Br. Mycol. Soc.* **5** 168 (1915)
Placed as a synonym of *D. toruloides* (Corda) Gueguen by Damon (1952)
4) *D. campaniforme* Mats., *Icones Fung. a Matsushima Lect.* 59 (1975)
An acceptable species.
5) *D. castaneum* G. Frag., *Bol. Soc. Brot.* ser. 2, **2** 46 (1924)
The dictyosporous solitary brown conidia formed in effuse colonies from short conidiophores place this species in *Monodictys* Hughes, probably as a synonym of *M. paradoxa* (Corda) Hughes.
6) *D. circinatum* Cke & Harkn., *Grevillea* **12** 95 (1883)
Damon (1952) excluded this from *Dictyosporium* and found the species to be the same as *Sporidesmium inquinans* Ellis and Everhart (1886). It is not a *Sporidesmium* species either, but shows strong similarities with the helicosporous hyphomycetes. The brown, euseptate, helical conidia, tightly coiled to eventually become like dictyospores, constricted at the septa, and formed holoblastically from a repent mycelium (figure 5) is typical of *Helicorhoidion* Hughes. The new combination ***Helicorhoidion circinatum*** (Cke & Harkn.) Sutton is proposed. Material examined: on *Platanus*, leg. Harkness no. 2434, holotype of *Dictyosporium circinatum* Cke & Harkn., IMI 722 ex K.
7) *D. coccophilum* Batista, *Bot. Agric. Pern.* **18** 5 (1951)
An acceptable species.

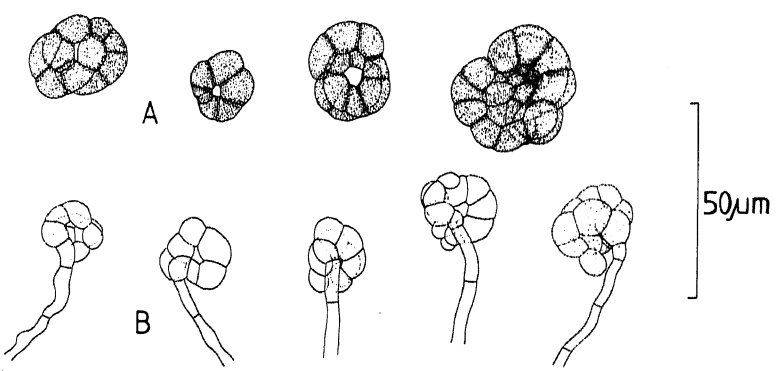


Figure 5. *Helicorhoidion circinatum*, holotype. **A.** conidia; **B.** developing conidia.

8) *D. crustaceum* (Karst.) Hughes, *Can. J. Bot.* **36** 762 (1958)
Hormiscium crustaceum Karst., *Medd. Soc. Fl. Fauna Fenn.* **14** 90 (1887)
9) *D. elegans* Corda in Weitenweber's *Beitrage* **1** 87 (1836)
Type species of the genus.
10) *D. foliicola* P. M. Kirk apud Kirk & Spooner, *Kew Bull.* **38** 583 (1984)
An acceptable species.
11) *D. heptasporum* (Garov.) Damon, *Lloydia* **15** 118 (1952)
An acceptable species
12) *D. hymenaearum* Batista & Bezerra, *Publ. Inst. Mic. Univ. Recife* **299** 24 (1960)
The original account and illustration indicate that this species may be based on no more than vegetative mycelium of an ascomycete or dematiaceous hyphomycete. No detached spores were figured. Doubtfully an acceptable species of *Dictyosporium.*
13) *D. intermedium* Subram., *Hyphomycetes:* 478 (1971), nom. invalid.
This name was not provided with a Latin description. It was interpreted as a small-spored *D. heptasporum.* Several collections of this species are now in herb. IMI and it seems opportune to provide a valid name for the species. In doing so I am delighted to dedicate the species to Prof. C V Subramanian who as a friend, fellow mycologist and humanitarian, has enriched not only my own existence but also that of many others by the qualities he has shown in his own life.

***Dictyosporium subramanianii* sp. nov.**

D. intermedium Subram., *Hyphomycetes*: 478 (1971), non rite publ.

A *D. heptasporum* differt conidiis minoribus 33–42 × 16–20 × 16–21 μm.

Icon. ibid. p. 479.

In lignis *Eucalypti globuli*, Jabalpur, India, N. D. Sharma, comm. 20 Sept. 1978, IMI 232361, holotypus; *Ficus*, Delhi, India, G. Mehrotra, 8 Nov. 1973, IMI 184005; *Holopteliae integrifoliae*, J. N. Agricultural University, Jabalpur, India, N. D. Sharma, comm. 31 July 1980, IMI 250191; *Psidii guajavae*, Gorakhpur, India, P. C. Misra, 7 Jan. 1975, IMI 225881; indet., Gorakhpur India, P. C. Misra, 1 Feb. 1981, IMI 260823.

14) *D. micronesicum* Mats., *Mycol. Mem.* **2** 8 (1981)
An acceptable species.
15) *D. minor* (Sacc.) Damon, *Lloydia* **15** 118 (1952)
Speira minor Sacc., *Michelia* **2** 559 (1882)
Placed as a synonym of *Cryptocoryneum bigeminum* (Cke & Ell.) Hughes (1958) but later included in the synonymy for *Eversia subopaca* (Cke & Ell.) Crane & Schoknecht by Schoknecht and Crane (1977).
16) *D. minus* Sacc., *Bull. R. Ort. bot. Napoli*: **34** (1918)
17) *D. oblongum* (Fuckel) Hughes, *Can. J. Bot.* **36** 762 (1958)
Speira oblonga Fuckel, *Jb. nassau. Ver. Naturk.* **23–24** 349 (1870)
An acceptable species.
18) *D. opacum* Cke & Harkn., *Grevillea* **12** 95 (1884)
Included as a synonym of *D. elegans* Corda, type species of the genus, by Damon (1952).
19) *D. pelagicum* (Linder) G. C. Hughes ex Gareth Jones, *Trans. Br. mycol. Soc.* **46** 137 (1963)
Speira pelagica Linder, *Farlowia* **1** 407 (1944)

Damon (1952) was of the opinion that this species did not belong in *Dictyosporium*. He preferred to place it in the '*Coniosporium—Sporidesmium*' complex. I am inclined to refer the species to *Monodictys* Hughes but this epithet cannot be transferred to this genus because of the existence of *M. pelagica* (Johnson) Gareth-Jones which is quite a different species.

20) *D. polystichum* (Höhn.) Damon, *Lloydia* **15** 118 (1952)
Speira polystichum Höhn., *Sber. Akad. Wiss. Wien* **122** 49 (1913)
An acceptable species.

21) *D. prolificum* Damon, *Lloydia* **15** 119 (1952)
An acceptable species.

22) *D. schizostachyfolium* Batista & Farr, *Saccardoa* **1** 103 (1960)
An acceptable species.

23) *D. secalinum* Delacr., *Bull. Soc. mycol. Fr.* 7 109 (1891)
This name was tentatively included in the synonymy for *D. elegans* Cda, by Damon (1952).

24) *D. toruloides* (Corda) Gueguen, *Bull. Soc. mycol. Fr.* **21** 98 (1905)
Speira toruloides Corda, *Icon. fung.* **1** 9 (1837)
An acceptable species.

25) *D. triseriale* Mats., *Mycol. Mem.* **1** 32 (1980)
An acceptable species.

26) *D. yerbae* Speg., *An. Mus. Nac. Buenos Aires* **12** 138 (1909)
Placed as a synonym of *Coniosporium paradoxum* (Corda) Mason & Hughes by Damon (1952), now known as *Monodictys paradoxa* (Corda) Hughes.

27) *D. zeylanicum* Petch, *Ann. R. Bot. Gdn Perad.* **6** 252 (1917)
An acceptable species.

7. *Digitodesmium*

Digitodesmium Kirk (1981) is also relevant to any discussion concerning *Cheiromyces*. It was introduced with a single species, *D. elegans* Kirk, described from rotten wood collected in the UK. The conidiomata are sporodochial, conidiogenesis is holoblastic and conidiogenous cells only produce a single conidium. The conidia were described as digitate with (2–)3–4(–6) slightly divergent arms. In these features *Digitodesmium* resembles *Cheiromyces* very closely, but conidial cross walls were described as of the euseptate type whereas in *Cheiromyces* they are distoseptate. It is on this feature that the two genera can only be distinguished. However the conidial cells in *Digitodesmium* have reduced lumina and the transverse walls do not fully abut onto the periclinal walls, both features being characteristic of distosepta. I hesitate to formally reduce *Digitodesmium* to synonymy with *Cheiromyces* even though the evidence indicates that this should probably be the course. The distinctions are fine, and may only be a reflection of two extreme ends of variation known to exist in other distoseptate taxa.

Material examined: on *Quercus robur*, Esher Common, Surrey, UK, P. M. Kirk, 5 June 1979, IMI 238430*e*, holotype; *Fagus sylvatica*, Esher Common, Surrey, UK, P. M. Kirk, 16 Sept. 1979, IMI 241392; ?*Taxus*, Thwaite Fall, Cottingham, Yorks. U.K., J. Webster, 8 Feb. 1948, IMI 23297*d*.

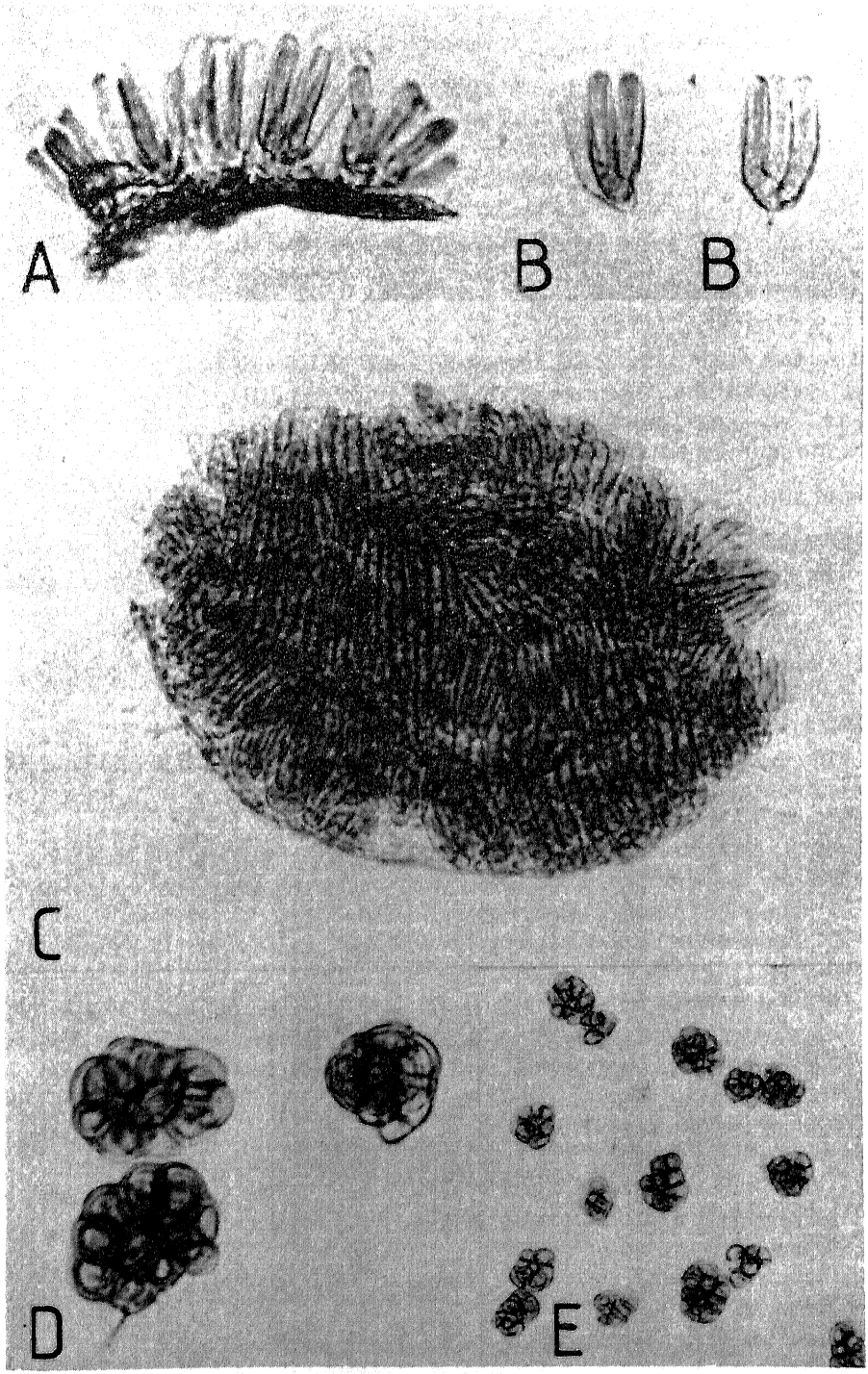


Figure 6. A–C. ***Cheiromyces stellatus.*** **A.** vertical section of conidioma showing attached conidia; **B.** separate conidia; **C.** whole conidioma mounted and slightly squashed. **D–E.** ***Helicorhoidion circinatum,*** conidia. (A, B × 1000; C × 400; D × 650; E × 250).

8. *Sirothecium*

Whereas the other genera considered here are all traditionally assigned to the hyphomycetes, *Sirothecium*, which was described by Karsten (1887) has been placed in the coelomycetes. The conidiomata are eustromatic and the cheiroid holoblastic conidia are formed from conidiogenous cells which line the walls of the conidiomata. The genus was fully described and illustrated by Sutton (1978, 1980) so only the formal synonymies are included below. Because the conidia were originally thought to be comprised of chains of cells the genus has become a repository of heterogeneous elements.

Sirothecium Karst., *Medd. Soc. Fauna Fl. Fenn.* **14** 105 (1887)
Cheiroconium Höhn., *Sber. Akad. Wiss. Wien* **119** 664 (June 1910)
Chelisporium Speg., *An. Mus. Nac. Buenos Aires* **20** 463 (Dec. 1910)
Description in Sutton (1978, 1980).

Species placed in *Sirothecium*.

1) *S. fragile* Morgan, *J. Mycol.* **10** 1 (1904)
The slender branched chains of spores described in the original account exclude this species from *Sirothecium*.
2) *S. globosum* Petch, *Ann. R. Bot. Gdn Peradeniya* **6** 235 (1917)
Conidia were described as pale fuscous, oval, 4–6 × 3–3·5 μm, features which exclude the species from *Sirothecium*.
3) *S. lichenicola* (Linds.) Keissl., *Ost. Bot. Zeit.* **60** 61 (1910)
Torula lichenicola Linds., *Trans. R. Soc. Edinb.* **25** 515 and 530 (1868–1869).
The name was transferred to *Vouauxiella* Petrak & Sydow by Petrak and Sydow (1927).
4) *S. lichenicola* forma *bispora* Keissl., *Zentbl. Bakt. ParasitKde* **27** 210 (1910)
Included as a synonym of *Lichenodiplis lecanorae* (Vouaux) Dyko & Hawksworth by Hawksworth and Dyko (1979).
5) *S. lichenicola* forma *cerinae* (B. de Lesd.) Keissl., *Zentbl. Bakt. ParasitKde* **27** 210 (1910)
Torula lichenicola forma *cerinae* B. de Lesd., *Bull. Soc. Bot. Fr.* **55** 424 (1858)
Questionably included as a synonym of *Lichenodiplis lecanorae* (Vouaux) Dyko & Hawksworth by Hawksworth and Dyko (1979) because the holotype was destroyed.
6) *S. minor* Sutton, *Kew Bull.* **33** 343 (1978).
An acceptable species.
7) *S. nigrum* Morgan, *J. Mycol.* **9** 82 (1903)
The branched chains of spores, readily falling apart, exclude this species from *Sirothecium*.
8) *S. saepiarium* Karst., *Medd. Soc. Fauna Fl. Fenn.* **14** 105 (1887)
Type species of the genus.
9) *S. tinctum* (Peck) Hughes, *Can. J. Bot.* **36** 805 (1958)
Cheiromyces tinctus Peck, *Bot. Gaz.* **5** 35 (1880)
Cheiroconium beaumontii Höhn., *Sber. Akad. Wiss. Wien* **119** 664 (June 1910)
Chelisporium hysterioides Speg., *An. Mus. Nac. Buenos Aires* **20** 463 (Dec. 1910)
An acceptable species.

10) *S. verrucosum* (Vouaux) Keissl., *Ost. Bot. Zeit.* **60** 62 (1910)
Torula verrucosa Vouaux, *Bull. trimestr. Soc. mycol. Fr.* **54** 697 (1907)

The name was transferred to *Vouauxiella* Petrak & Sydow by Petrak and Sydow (1927).

Acknowledgements

I am grateful to the Curators of herb. K and W for permission to examine material in their keeping. The material of *D. australiense* was collected during a visit to Australia partly funded by the Rural Credits Development Fund to which due acknowledgement is made.

References

Berkeley M J 1857 *Introduction to cryptogamic botany.* (London: Balliere)
Berkeley M J 1875 Notices of north American fungi; *Grevillea* **3** 97–112
Boerema G H, Dorenbosch M M J and van Kesteren H A 1973 Remarks on species of *Phoma* referred to *Peyronellaea*; *Persoonia* **7** 131–139
Carmichael J W, Kendrick W B, Conners I L and Sigler L 1980 *Genera of hyphomycetes* (Alberta: Univ. Press) pp. 1–386
Corda A C I 1836 Mykologische Beobachtungen. in Weitenweber, *Beitrage zur gesammten Natur- und Heilwissenschaften* (Prague)
Corda A C I 1837 *Icones fungorum hucusque cognitorum* (Prague: J. G. Calve) **1** 1–32
Damon S C 1950 A taxonomic consideration of two cheirosporous genera *Cheiromyces* and *Pedilospora*; *Mycologia* **42** 554–562
Damon S C 1952 Type studies in *Dictyosporium, Speira* and *Cattanea*; *Lloydia* **15** 110–124
Ellis J B and Everhart B M 1886 New species of fungi from various localities; *J. Mycol.* **2** 37–42
Ellis M B 1971 *Dematiaceous hyphomycetes.* (Kew: Commonwealth Mycological Institute)
Garovaglio S 1875 Di alcuni nuovi funghi parasiti trovate dall dottore Achille Cattaneo, allievo practicante a Laboratoria Crittogamico, sugli argumi affeti dal mal della gomma e dalla gangrena; *Rend. Ist. Real. Lombardi di Sci. e Lett.* **8** 118–126
Hawksworth D L and Dyko B J 1979 *Lichenodiplis* and *Vouauxiomyces*: two new genera of lichenicolous coelomycetes; *Lichenologist* **11** 51–61
Hawksworth D L, Sutton B C and Ainsworth G C 1983 *Dictionary of the fungi* 7th edn (Kew: Commonwealth Mycological Institute)
Höhnel F von 1903 Mycologische Fragmente; *Ann. Mycol.* **1** 391–414
Höhnel F von 1910 Fragmente zur Mykologie (XI. Mitteilung, Nr. 527 bis 573); *Sber. Akad. Wiss. Wien* **119** 617–679
Höhnel F von 1914 Verzeichnis der von mir gemachten Angahen zur Systematik und Synonomie der Pilze; *Ost. Bot. Z.* **63** 167–510
Höhnel F von 1923 System der Fungi Imperfecti Fuckel; *Mykol. Unters.* **1** 301–369
Hughes S J 1958 Revisiones hyphomycetum aliquot cum appendice de nominibus rejiciendis; *Can. J. Bot.* **36** 727–836
Karsten P A 1887 Symbolae ad Mycologiam fennicam XIX; *Medd. Soc. Fl. Fauna Fenn.* **14** 85–94
Kirk P M 1981 New or interesting microfungi II. Dematiaceous hyphomycetes from Esher Common, Surrey; *Trans. Br. Mycol. Soc.* **77** 279–297
Martin G W 1944 The fungus genus *Cheiromyces*, with description of a new species; *J. Wash. Acad. Sci.* **34** 358–360
Moore R T 1958 Deuteromycetes I: The *Sporidesmium* complex; *Mycologia* **50** 681–692
Morgan-Jones G, Nag Raj T R and Kendrick W B 1972 Icones generum coelomycetum V; *Univ. Waterloo Biol. Ser.* **7** 1–52
Petrak F and Sydow H 1927 Die Gattungen der Pyrenomyzeten, Sphaeropsideen und Melanconieen; *Beih. Rep. Spec. Nov. Regni Veg.* **42** 1–551

Rimington F C 1953 Fungi in *The natural history of the Scarborough district* **1** Geology and Botany 81–166
Saccardo P A 1886 *Sylloge Fungorum* **4** 1–807 Padua
Saccardo P A 1911 *Sylloge Fungorum* **20** 1–1310 Padua
Saccardo P A and Trotter A 1913 *Sylloge Fungorum* **22** 1–1612 Padua
Schoknecht J D and Crane J L 1977 Revision of *Torula* and *Hormiscium* species. *Torula occulta, T. diversa, T. elasticae, T. bigemina* and *Hormiscium condensatum* re-examined; *Mycologia* **69** 533–546
Spegazzini C 1910 Mycetes Argentinensis ser. 5; *An. Mus. Nac. Buenos Aires* **20** 329–467
Strauss F F von 1853 Sturm's *Deutschlands Flora* III Abt. Die Pilze Deutschlands **7** 1–48
Sutton B C 1978 Two undescribed deuteromycete microfungi from the Azores; *Kew Bull.* **33** 343–346
Sutton B C 1980 *The Coelomycetes.* (Kew: Commonwealth Mycological Institute)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 245–252.
© Printed in India.

On the anamorph genus *Cirrenalia*

R D GOOS
Department of Botany, University of Rhode Island, Kingston, Rhode Island, 02881, USA

Abstract. The taxonomic status of the anamorph genus *Cirrenalia* is reviewed. As presently constituted, the genus is comprised of five marine and five terrestrial species. Descriptions of the species are given and a dichotomous key to species provided.

Keywords. Helicosporous hyphomycetes; marine fungi; terrestrial fungi.

1. Introduction

The genus *Cirrenalia*, one of the genera of helicosporous Hyphomycetes, was erected by Meyers and Moore (1960) to accommodate the single species, C. *macrocephala* (Kohlmeyer) Meyers and Moore, a widespread fungus on wood in marine habitats. Kohlmeyer (1966, 1968) subsequently described three additional species from marine habitats, and a fifth species was described by Schmidt (1969), also from the marine environment. The early concept of *Cirrenalia* thus became one of a genus of marine fungi. Sutton (1973), in describing *C. donnae,* set aside ecological considerations and assigned a terrestrial species to the genus, basing his placement of the fungus on the mode of conidium formation and morphology of the conidium. Sutton's lead was followed by Rao and Reddy (1978), Matsushima (1980), Sugiyama (1981) and Kirk (1981), all of whom described new species of *Cirrenalia* based on fungi encountered in terrestrial habitats.

As presently constituted, *Cirrenalia* contains ten species, five of which are marine and five of which are terrestrial. The unifying characteristics are morphological and developmental, as is consistent with the current philosophy for classifying anamorphic (imperfect) fungi. The diagnostic features of the ten recognized species are summarized in table 1. Sutton (1973) pointed out that a distinctive feature of *Cirrenalia* is that the

Table 1. Diagnostic characteristics of the species of *Cirrenalia*.

Species	Conidial filament (Diam.) (μm)	Conidium size (μm)	Conidiophores	Condiophore size (μm)
C. donnae	10	20–25·5	on Sporodochia	–35 × 3–6
C. fusca	11–22·5	20–36 × 20–30	Micronematous	–33 × 2
C. indica	10–15	10–18	Micronematous	6–9 × 3–6
C. japonica	12–20	18–28	Fasciculate	–63 × 2·5–6
C. lignicola	5–6	15–20	Micronematous	– × 2 (diam.)
C. macrocephala	10–15	20–25	Semimacronematous	3·5–25 × 2–5
C. palmicola	5–8	40–100 × 5–8	Micronematous	– × 1–2·5
C. pseudomacrocephala	16–20	27–38	Semimacronematous	22–30 × 3–5
C. pygmea	16–23	25–31 × 28–34	Lacking	–
C. tropicalis	9–12	30–38	Macronematous	25–42 × 2·5–5

apex of the conidiogenous cell and the basal cell of the conidium are both at the center of the helix formed by the conidial filament, whereas in other helicosporous fungi, such as *Helicosporium* and *Helicoma*, they are found on the periphery of the helix. No teleomorphs (perfect states) have been described for any of the species of *Cirrenalia*. The purpose of this paper is to review the current status of the genus, to provide updated descriptions of similar format for the ten known species, and to present a key for their identification. A review of the marine species was given by Kohlmeyer and Kohlmeyer (1979) and a key to four species can be found in Ellis (1976).

2. Generic and species descriptions

Cirrenalia Meyers and Moore. 1960. American Journal of Botany, 47: 345–349
Emended J. Kohlmeyer, Berlin Deutches Botanishe Gessellschaft 79: 35. 1966

Colonies pulvinate and punctiforme or effuse, reddish brown to black. *Mycelium* superficial or immersed. *Conidiophores* micronematous or semi-macronematous, sometimes with inflated cells, or lacking. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, sometimes inflated. *Conidia* helicoid or irregularly coiled, acrogenous, solitary, dry, septate, sometimes constricted at the septa, brown, often with some cells darker than others, smooth. Teleomorphs unknown.

2.1 *Cirrenalia donnae* Sutton. 1973. Commonwealth Mycological Institute Mycological Paper 132: 32–36

Sporodochia scattered, punctiform, dark reddish brown, loose, shining, up to 100 μm diameter. *Mycelium* immersed or superficial, composed of branched, septate, brown hyphae up to 3 μm wide. *Stroma* none or only rudimentary. *Conidiophores* fasciculate, simple or branched, pale brown, septate, smooth, often formed of inflated cells and markedly constricted at the septa, up to 35 μm long × 3–6 μm wide. *Conidiogenous cells* monoblastic, integrated, terminal, determinate. *Conidia* acrogenous, solitary, dry, helicoid, smooth-walled, reddish brown with the basal cell somewhat lighter, 7–11 septate, sometimes with a longitudinal or oblique septum, apex obtuse, base truncate, 20–25·5 μm diameter; *filament* 10 μm diameter at the apex, becoming progressively narrower towards the base, which is 5 μm wide.

Substrate: On bark of *Abies balsamea*; on bark of *Sorbus*.

Distribution: Canada (Manitoba, Saskatchewan); England.

2.2 *Cirrenalia fusca* Schmidt. 1969. Repertorium Specierum Novarum Regni Vegetabilis 80: 110–112

Mycelium immersed and superficial, composed of branched, septate, yellow or brown hyphae, (1–) 2·5–3·3 μm in width. *Conidiophores* micronematous, hypha-like, 1–2 septate, up to 33 μm long and 2 μm in width. *Conidiogenous cells* integrated, monoblastic, terminal, determinate. *Conidia* solitary, brown to dark-brown, more or less helicoid, 2–4 septate, increasing in diameter and pigmentation from base to apex; spirals 20–36 × 20–30 μm in size, terminal cell 13–33 μm long by 11–22·5 μm broad.

Substrate: Dead rhizomes of *Phragmites communis* and unidentified reeds.

Distribution: Baltic Sea, Germany; Mediterrean, France.

2.3 *Cirrenalia indica* V Rao and A Reddy. 1978. Indian Journal of Mycological Research 16: 306–308

Colonies effuse, thin, granular, blackish brown. *Mycelium* partly superficial, partly immersed, composed of branched, septate, subhyaline, smooth hyphae up to 3 μm wide. *Conidiophores* micronematous or semi-macronematous, with 0–3 septa, smooth, subhyaline, simple, 6–9 μm long, 3–6 μm in diameter. *Conidiogenous cells* monoblastic, integrated, terminal, determinate. *Conidia* acrogenous, solitary, dry, mostly curved, 2–5 celled, the terminal cell conspicuously large, smooth, subhyaline at the base, pale to dark brown at the apex, apical cell variable in shape, 10–18 μm in diameter; *filament* 10–15 μm in diameter.

Substrate: On dead wood.

Distribution: India

2.4 *Cirrenalia japonica* Sugiyama. 1981. Transaction of the Mycological Society of Japan 22: 47–48

Colonies scattered, pulvinate, punctiform, dark reddish brown to blackish brown, loose, shining, up to 250 μm in diameter. *Mycelium* immersed or superficial, composed of branched, septate, thick-walled brown hyphae, 2·5–5·5 μm wide. *Stroma* rudimentary or none. *Conidiophores* fasciculate, simple or branched, pale brown, septate, smooth, up to 63 μm long, 2·5–6 μm wide, often with inflated cells up to 12 μm wide. *Conidiogenous cells* monoblastic, integrated, terminal, determinate, often inflated. *Conidia* acrogenous, solitary, dry, tightly coiled at maturity 1/2–1 time, smooth, reddish brown, with 4–9 transverse septa and mostly one or more longitudinal and/or oblique septa, not or slightly constricted at the septa, apex obtuse, basal cell pale brown and conico-truncated; semi-coiled conidia commonly 18–28 μm in diameter; the *filament* 12–20 μm wide at the broadest part (or at the apex).

Substrate: On cortex of *Abies homolepis*.

Distribution: Japan

2.5 *Cirrenalia lignicola* Kirk. 1981. Transactions of the British Mycological Society 77: 283

Colonies scattered, minute, inconspicuous, punctiform, dark brown, up to 50 μm wide. *Mycelium* partly superficial, partly immersed in the substratum, composed of branched, septate, smooth, brown hyphae up to 3 μm wide. *Conidiophores* micronematous, mononematous, solitary or fasciculate, simple or branched, very pale brown, smooth, septate, up to 2 μm wide. *Conidiogenous cells* monoblastic, integrated, terminal, determinate. *Conidia* acrogenous, solitary, dry, helicoid, smooth, olivaceous brown, septate, 15–20 μm in diameter, 5–6 μm in width.

Substrate: On rotten bark and wood of *Fagus sylvatica* and other trees.

Distribution: England.

2.6 *Cirrenalia macrocephala* (Kohlmeyer) Meyers and Moore. 1960. American Journal of Botany 47: 346–348
≡ *Helicoma macrocephala* Kohlmeyer, 1958

Colonies effuse, thin, black, granular, composed of septate, branched, hyaline to dark brown hyphae, 1·5–2 μm in diameter. *Conidiophores* narrow, cylindrical, occasionally apically inflated, 0–3 septate, simple, hyaline to yellowish, 3·5–25 μm in length, 2–5 μm in diameter. *Conidiogenous cells* monoblastic, integrated, terminal, determinate. *Conidia* mostly coiled 1–1¼ times, 2–7 but mostly 4 septate, strongly constricted at the septa, cells increasing in diameter and pigmentation from base to apex; coiled conidia 12–35 × 12–23·5, commonly 20–25 μm in diameter, the *filament* 10–15 μm in diameter in the widest part.

Substrate: Wood exposed to sea water.

Distribution: Widespread, particularly in temperate waters. (See comment in Kohlmeyer (1979) regarding reports from tropical waters.)

2.7 *Cirrenalia palmicola* Matsushima. 1980. Matsushima Mycological Memoirs No. 1, page 17

Colonies on V-8 agar slow growing, fuscous. *Conidiophores* micronematous, pale brown, echinulate, 1–2·5 μm in diameter. *Conidiogenous cells* monoblastic, integrated. *Conidia* formed acrogenously, solitary, dry, elongate, cylindrical, helically coiled or irregularly contorted, 40–100 μm long and 5–8 μm in diameter, 10–30 septate, dark brown.

Substrate: Dead stem of *Cocos nuciferae*.

Distribution: Taiwan.

2.8 *Cirrenalia pseudomacrocephala* Kohlmeyer. 1968. Mycologia 60: 66

Colonies effuse, thin. *Mycelium* immersed or superficial, composed of brown, septate, hyphae, 2·5–5 μm in diameter. *Conidiophores* cylindrical, simple, straight or curved, 0–2 septate, 23–30 × 3–5 μm in size. *Conidiogenous cells* monoblastic, integrated, terminal. *Conidia* mostly coiled, 3–5 septate, slightly or strongly constricted at the septa, fuscous to grayish-brown, the swollen terminal and subterminal cells darker than the others, septa thick and very dark; up to 50 μm long when not coiled, coiled conidia 27–38 μm in diameter; *filament* 16–20 μm in the widest part.

Substrate: Driftwood under mangroves.

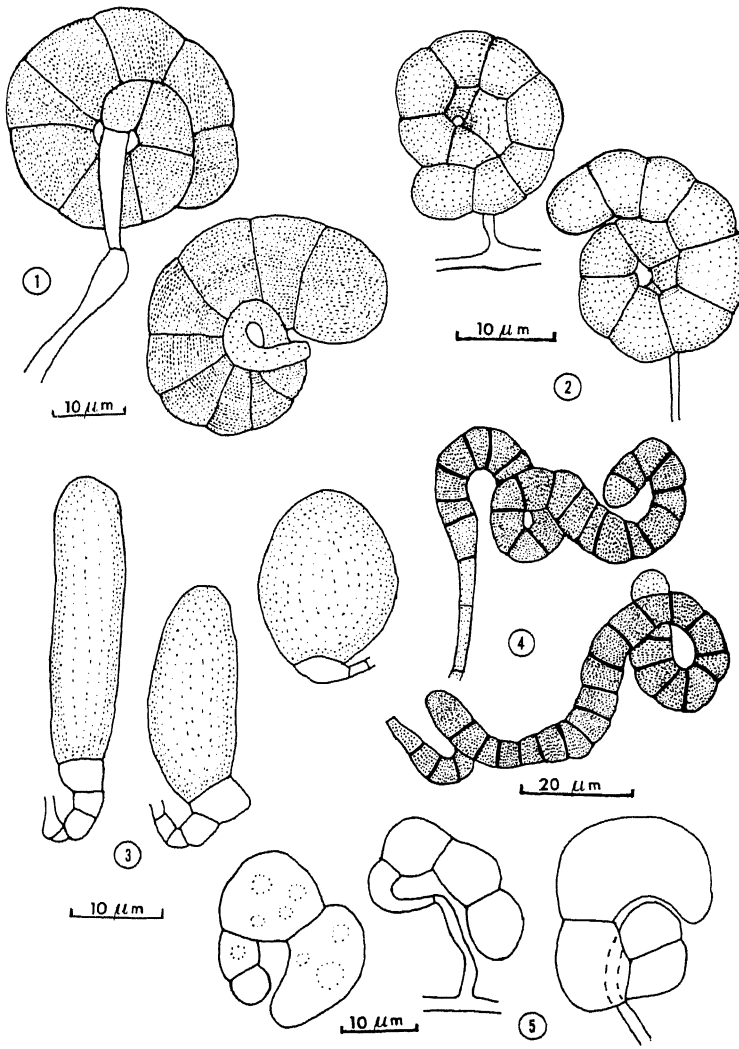
Distribution: Bermuda, Mexico.

2.9 *Cirrenalia pygmea* Kohlmeyer. 1966. Berlin Deutsches Botanish Gesellshaft 79: 35

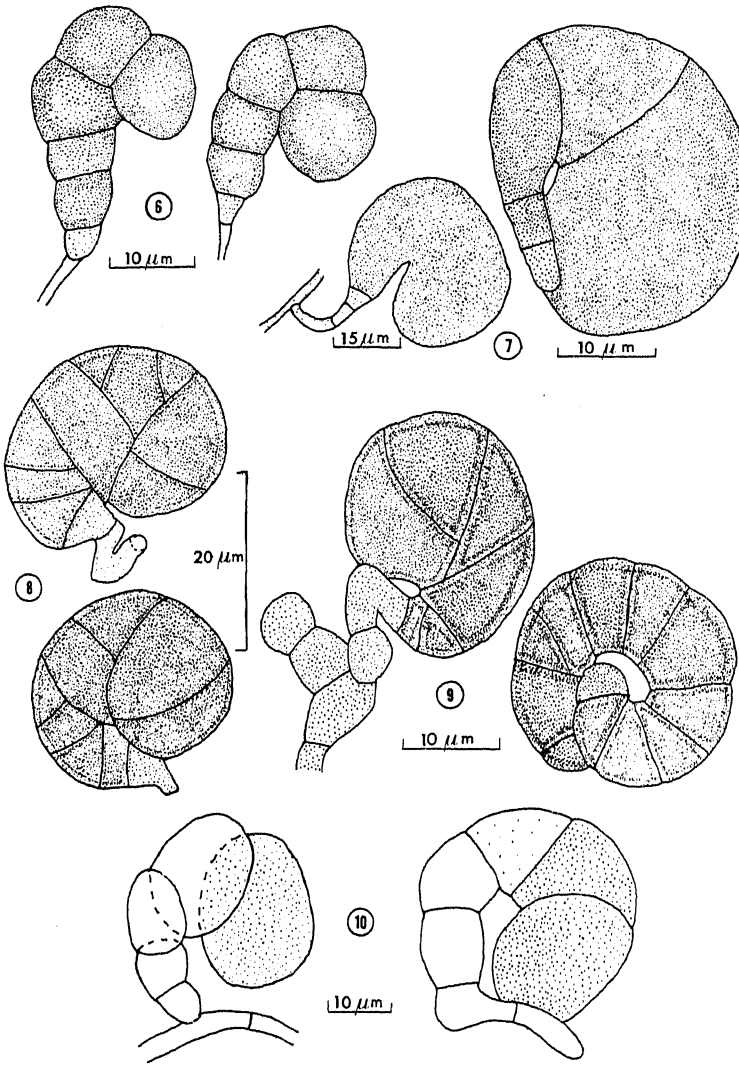
Colonies effuse, thin. *Mycelium* composed of septate, branched, fuscous hyphae 2·2–4·5 μm in diameter. *Conidiophores* lacking. *Conidia* developing as lateral outgrowths from the mycelium, solitary, helicoid, coiled 1/2–1 time, 3–4 septate, not or only slightly constricted at the septa, fist-shaped or reniform, black or fuscous; cells increasing in diameter from base to apex; coiled conidia 25·5–31 × 28·5–34 μm; terminal cell 16·5–23 μm in diameter, subglobose to reniform, basally flattened.

Substrate: Bark and wood of dead roots of *Mangifera indica, Rhizophora mangle,* and *R. racemosa*; driftwood.

Distribution: Liberia, Trinidad, Mexico (Chiapas).



Figures 1–5.



Figures 1–10. Conidia of *Cirrenalia* species, redrawn from sources indicated. **1.** *C. tropicalis* (Kohlmeyer 1968), **2.** *C. lignicola* (Kirk 1981), **3.** *C. indica* (Rao and Reddy 1978), **4.** *C. palmicola* (Matsushima 1980), **5.** *C. fusca* (Schmidt 1969), **6.** *C. macrocephala* (Kohlmeyer 1958), **7.** *C. pygmea* (Kohlmeyer 1966, 1968), **8.** *C. japonica* (Sugiyama 1981), **9.** *C. donnae* (Sutton 1973), **10.** *C. pseudomacrocephala* (Kohlmeyer 1968).

2.10 *Cirrenalia tropicalis* Kohlmeyer. 1968. Mycologia 60: 267

Colonies effuse, thin, granular, blackish brown. *Mycelium* superficial or immersed, composed of light brown, septate hyphae 2·5–5 µm in diameter. *Conidiophores* pale brown, cylindrical, twisted, 0–4 septate, 25–42 × 2·5–5 µm. *Conidiogenous cells* monoblastic, integrated, terminal, determinate. *Conidia* regularly or irregularly helicoid, mostly coiled 1–1½ times, 6–12 septate, not or only slightly constricted at the

septa, reddish brown to dark brown, 30–38 μm in diameter, terminal cell 9–12 × 10–20 μm.

Substrate: Intertidal wood; prop roots of *Rhizophora racemosa*; calcareous linings of empty shipworm tubes.

Distribution: Liberia.

3. Key to the species of *Cirrenalia*

1. Conidiophores fasciculate or formed on sporodochia 2
1. Conidiophores not fasciculate 3
   2. Conidiophores on sporodochia; conidia 7–11 septate, 20–25 μm in diam., filament 10–11 μm at widest point. ..... *C. donnae*
   2. Conidiophores fasciculate; conidia 4–9 septate, tightly coiled, 18–28 μm in diameter, filament 12–20 μm in diameter ..... *C. japonica*
3. Conidial filament not exceeding 8 μm 4
3. Conidial filament greater than 8 μm 5
   4. Conidia loosely helicoid, consisting of an irregularly contorted filament with 10–30 septa, 5–8 μm in diam., and 40–100 μm in length ..... *C. palmicola*
   4. Conidia distinctly helicoid, tightly coiled, filament 5–6 μm; conidia 15–20 μm diameter. ..... *C. lignicola*
5. Conidial filament at least 15 μm or more in diameter 6
5. Conidial filament not consistently more than 15 μm in diameter 7
   6. Conidiophores lacking; conidial filament 16–23 μm; conidia 25–31 × 28–34 . ..... *C. pygmea*
   6. Conidiophores cylindrical, simple, 23–30 × 3–5 μm; conidial filament 16–20 μm; conidia 27–38 μm in diameter ..... *C. pseudomacrocephala*
7. Conidia coiled 1–1½ times, 6–12 septate, 30–30 μm in diameter. ... *C. tropicalis*
7. Conidia generally of smaller size 8
   8. Terminal cell of conidium conspicuously large, pale to dark brown, 10–18 μm in diameter ..... *C. indica*
   8. Terminal cell of conidium not conspicuously enlarged 9
9. Conidia reddish-brown, 2–7 septate, strongly constricted at the septa; coiled conidia mostly 20–25 μm in diameter; filament 10–15 μm ..... *C. macrocephala*
9. Conidia without reddish tint, 2–4 septate, not markedly constricted at septa, 20–36 μm in diameter; filament 11–22 μm broad. ..... *C. fusca*

4. Discussion

The genus *Cirrenalia* appears to represent a heterogenous assemblage of fungi. All species assigned to the genus have a similar mode of conidium ontogeny, but morphology of the mature conidium and the diverse biology of the group strongly suggest that this is a disparate group of fungi. It may well be that the genus should be retained for the marine species, which seem, on the whole, to form a more natural group. Reassignment of the terrestrial species in the absence of additional information regarding natural relationships would be premature, however, so for the present, all

species presently assigned to the genus are retained. A knowledge of teleomorphs (sexual states) is essential for meaningful reassignment.

References

Ellis M B 1976 *More dematiaceous hyphomycetes* (Kew, Surrey, England: Commonwealth Mycological Institute) 507 pp.

Kirk P M 1981 New or interesting microfungi II Dematiaceous hyphomycetes from Esher Common; *Surrey Trans. Br. Mycol. Soc.* 77 267–271

Kohlmeyer J 1958 Beobactungen uber mediterrane Meerespilze sowie das vorkommen von marinen Moderfaule-Erregern in Aquariumszuchten holzzerstorender Meerestiere; *Ber. Dtsch. Bot. Ges.* **71** 98–116

Kohlmeyer J 1966 Neue Meerespilze an Mangroven Berlichten der Deutschen; *Bot. Ges.* **79** 27–37

Kohlmeyer J 1968 Marine fungi from the tropics; *Mycologia* **60** 252–270

Kohlmeyer J and Kohlmeyer E 1979 *Marine mycology The higher fungi* (New York and London: Academic Press) 690 pp.

Matsushima T 1980 *Saprophytic microfungi from Taiwan Part I* Matsushima mycological memoir No. 1 Published by the author Kobe Japan.

Meyers S and Morre R T 1960 Thalassiomycetes II New Genera and species of Deuteromycetes; *Am. J. Bot.* **47** 345–349

Rao V and K A Reddy 1978 Some new microfungi from India; *Indian J. Mycol. Res.* **16** 301–309

Schmidt I 1969 *Carbosphaerella pleosporoides* und *Cirrenalia fusca; Feddes Rep.* **80** 107–112

Sugiyama J 1981 Microfungi Japonicae I Nova species *Cirrenalia* in Cortice *Abietis homolepsis; Trans. Mycol. Soc. Jpn* **22** 47–53

Sutton B C 1973 *Hyphomycetes from Manitoba and Saskatchewan* CMI Mycol PAP 132 143 pp.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 253–257.
© Printed in India.

Porosubramaniana, a new genus of lignicolous Hyphomycetes

VĚRA HOLUBOVÁ-JECHOVÁ

Botanical Institute, Czechoslovak Academy of Sciences, 252 43 Průhonice near Praha, Czechoslovakia

Abstract. Porosubramaniana Hol.-Jech., a new genus of porosporous dematiaceous Hyphomycetes is described to accommodate a new species *P. moniliformis* Hol.-Jech. occurring on decayed wood in Poland.

Keywords. Porosubramaniana gen. nov.; *Porosubramaniana moniliformis* sp. nov.; lignicolous Hyphomycetes; Dematiaceae.

1. Introduction

During the studies on the lignicolous hyphomycete flora of Central Europe, pursued by the present author, several collections of interesting fungi were found. Among them an unusual fungus unassignable to any genus known to the author was encountered. It was collected in 1973 in the virgin forest Bialowieża, in the north-eastern part of Poland. The taxon is related by the pattern of its conidiogenesis to the lignicolous and herbicolous genus Piricauda Bubák, from which it differs in such significant characteristics as macronematous, polytretic conidiophores and 1-septate conidia; also the presence of a synanamorph "Selenosporella" is an interesting feature of this taxon.

In honour of the excellent hyphomycetologist of this time, Prof. C V Subramanian, the new genus named Porosubramaniana is proposed here to accommodate the taxon mentioned above.

2. Taxonomic part

Porosubramaniana Hol.-Jech. gen. nov.

Fungi imperfecti, Hyphomycetes, Dematiaceae.

Coloniae effusae, brunneae vel atrobrunneae.

Mycelium plerumque superficiale et partim immersum in substrato, ex hyphis ramosis, septatis, brunneis levibusque compositum. Stroma abest. Setae et hyphopodia nulla.

Conidiophora macronemata, mononemata, simplicia vel irregulariter ramosa, ascendentia, flexuosa vel recta, pallide brunnea vel brunnea, levia, septata, moniliformiter constricta. Cellulae conidiogenae mono- vel polytreticae, in conidiophoris incorporatae, intercalares, doliiformes, cicatricatae.

Conidia singula, sicca, pleurogena, late ellipsoidea, ovoidea vel late navicularia, leviter rostrata, 1-septata, brunnea vel atrobrunnea, crassitunicata, asperata vel verruculosa.

Synanamorpha: Selenosporella

Colonies effuse, brown to blackish brown.

Mycelium mostly superficial, partly immersed in the natural substratum, composed of loosely branched, septate, brown, smooth hyphae. Stroma none. Setae and hyphopodia absent.

Conidiophores macronematous, mononematous, simple or occasionally irregularly branched, ascending, flexuous or straight, pale brown to brown, smooth, septate, moniliform constricted. Conidiogenous cells mono- or polytretic, integrated, intercalary, barrel-shaped, cicatrized, with distinct pores in the middle of dark prominent scars.

Conidia solitary, dry, pleurogenous, broadly ellipsoidal, ovoid or broadly navicular, slightly rostrate, 1-septate, brown to dark brown, thick-walled, roughened to verruculose.

2.1 *Species typica*

Porosubramaniana moniliformis Hol.-Jech. spec. nova. Figure 1.

Coloniae effusae, pulveraceae vel velutinae, brunneae vel atrobrunneae.

Mycelium plerumque superficiale, ex hyphis pallide brunneis vel brunneis, septatis, 2–2·5 (–3) μm latis, laxe ramosis crassitunicatisque compositum.

Conidiophora singula, repentia, ascendentia, vel interdum erecta, flexuosa vel recta, subcylindrica, simplicia vel irregulariter ramosa, ad basin pallide brunnea vel brunnea, ad apicem subhyalina, usque 12-septata, constricta, 30–130 μm longa, 4–5·5 μm lata, ad apicem elongascentia. Cellulae conidiogenae mono- vel polytreticae, intercalares, interdum terminales, doliiformes, cicatricatae.

Conidia late ellipsoidea, ovoidea vel late navicularia, leviter rostrata, 1-septata, brunnea vel atrobrunnea, crassitunicata, asperata vel verruculosa, (12–) 14–19 (–20·5) × 7–9 μm, ad basin rotundata, ad apicem papillata, pallidiora et tenuitunicata.

Synanamorpha "Selenosporella" in conidiophoris diversis, non ramosis vel verticillariter ramosis oriunda; cellulae conidiogenae lageniformes, sympodiales, polyphialidicae, 30–45 μm longae; conidia aseptata, filiformes, hyalina, tenuitunicata, 8–12 × 0·5 μm.

Habitat in ligno putrido trunci emortui deiecti Carpini betuli.

Holotypus: Polonia septentr.- orientalis: in silva virginea "Puszcza Bialowieska", loco Park Narodowy (*ca* 160 m s.m.) (quadratum no. 398), distr. Hajnówka; ad truncum putridum deiectum Carpini betuli, 24.VIII.1973, leg. V. Holubová-Jechová (PRM 836570).

Colonies effuse, powdery to velvety, brown to dark blackish brown.

Mycelium mostly superficial, composed of pale brown to brown, septate, 2–2·5 (–3) μm wide, loosely branched, thick-walled hyphae, with scattered small thickenings of the wall.

Conidiophores arising singly from basal hyphae, repent or ascending or occasionally erect, flexuose or straight, subcylindrical, simple or irregularly branched, pale brown to brown near the base, subhyaline towards the apex, up to 12-septate, constricted at septa, 30–130 μm long, 4–5·5 μm wide, successively prolongated at the apex. Conidiogenous cells mono- or polytretic, intercalary, exceptionally terminal, barrel-shaped, each with a distinct pore in the middle of 1–3 dark, thick-walled, conidial scars.

Conidia broadly ellipsoidal, ovoid to broadly navicular, slightly rostrate, 1-septate, brown to dark brown, thick-walled, roughened to verruculose, (12–) 14–19

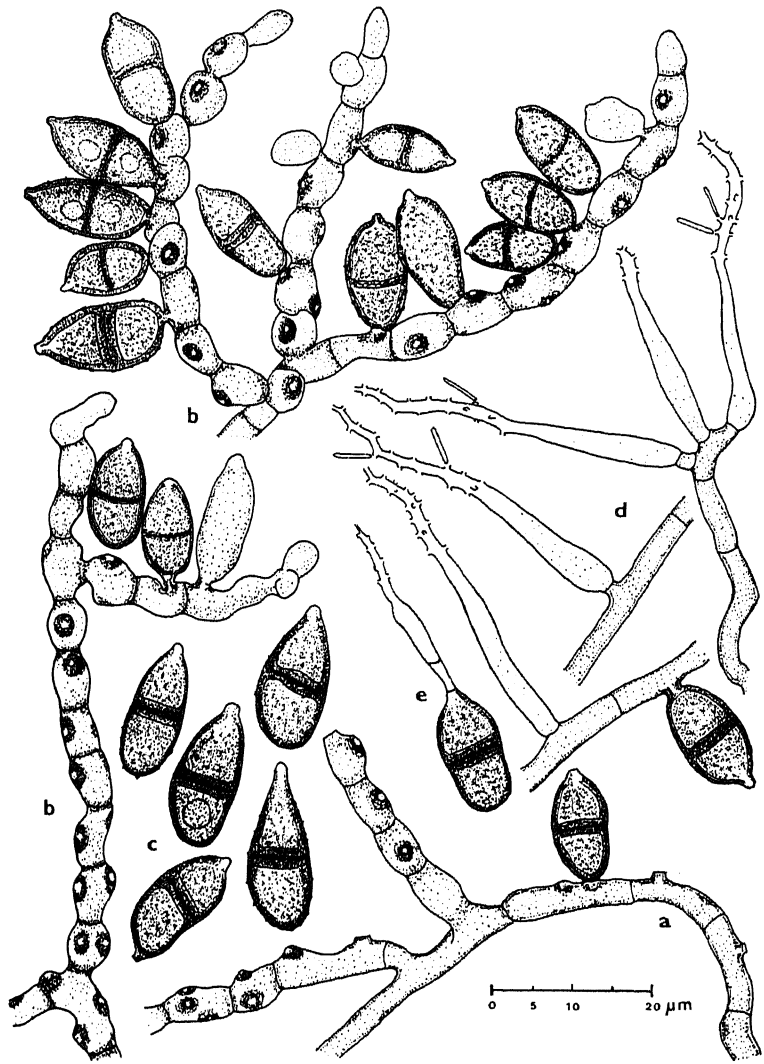


Figure 1. *Porosubramaniana moniliformis* Hol.-Jech. sp. nov. – **a.** basal hypha with arising conidiophores and one conidium, **b.** branched conidiophores with lateral conidia, **c.** mature conidia, **d.** conidiophores and conidia of "Selenosporella" synanamorph, **e.** germinating conidium forming a Selenosporella-like polyphialide.

(–20·5) × 7–9 µm, the basal part rounded or occasionally slightly prominent, the apical part papillate, paler and thin-walled, evidently bearing a germ pore. Conidia borne occasionally also on basal repent hyphae.

Synanamorph "Selenosporella" borne on separate conidiophores which are simple or verticilatelly branched, pale brown, smooth, 2–3 µm wide; conidiogenous cells lageniform, sympodial, polyphialidic, 30–45 µm long, 1·5–2 µm wide in the apical part, with protruding denticular collarettes (this part 10–20 µm long); conidia filiform, straight or slightly curved, hyaline, thin-walled, aseptate, 8–10 µm long, 0·5 µm wide.

Conidiophores of Selenosporella-like synanamorph arise from the same basal hyphae as conidiophores of *Porosubramaniana moniliformis*. A few conidia were observed germinating in a conidiogenous cell of the synanamorph, too.

The fungus was collected on two different but close localities in the area Park Narodowy "Bialowieża" on dead rotten trunk and dead rotten branch of Carpinus betulus.

2.2 *Material studied*

Polonia septentr.- orientalis: in silva virginea "Puszcza Bialowieska", loco Park Narodowy (*ca* 160 m s.m.) distr. Hajnówka, 1) quadratum no. 398, ad truncum putr. deiect. Carpini betuli, 24.VIII.1973, leg. V. Holubová-Jechová (PRM 836570 – Holotypus); 2) quadratum no 370, ad ramum putr. deiect. Carpini betuli, 26.VIII.1973, leg. V. Holubová-Jechová (PRM 836569).

3. Discussion

The new species of a lignicolous dematiaceous hyphomycete described and classified here in the new genus Porosubramaniana has very distinct conidiogenesis and morphology of its conidiophores. Conidiophores arise singly from basal hyphae and elongate apically; they are acroauxic. Its growth is not terminated by the production of a conidium, but on the contrary, the apical part of the conidiophore (1–3 apical cells) is sterile. No terminal conidium was observed. The conidiogenous cells are integrated and intercalare in the basal and middle part of the conidiophore. Conidia are borne singly and pleurogenously, one or three conidia on one conidiogenous cell. Sometimes a conidiogenous point may be located directly on the basal hypha, so one or more conidia are borne on it. Conidiogenesis is blastic, obviously enteroblastic. The wall of the conidiogenous cell around the budding locus is thickened and melanized. After secession of a conidium a rounded scar remains with a slightly prominent pore in the middle and a margin of darkened wall around. It is not easy to ascertain by light microscopy whether conidia arise as protrusions through pores dissolved in the wall of the conidiogenous cell or whether they develop holoblastically. As the conidiogenous scars have darkened and thickened walls with a light pore in the middle the opinion is accepted here that conidia originate through pores (by the "porosporous" conidiogenesis). Succession of conidia was observed; the youngest conidia are the nearest to the terminal part of the conidiophore. The conidia on conidiogenous cells are formed laterally, not sympodially as it is common in some porosporous dematiaceous genera, as in Drechslera Ito, Exosporium Link, Curvularia Boedijn, Ulocladium Preuss etc. Younger conidia are paler with a thinner wall, mature conidia are thick-walled, dark brown and verruculose. Conidial initials are at first subspherical and subhyaline to hyaline, then somewhat ovoid or ellipsoidal and subhyaline to pale brown; they soon become septate.

By its conidiogenesis Porosubramaniana seems to be related to Piricauda Bubák, which is known as the parasitic or saprophytic hyphomycete from tropical and subtropical regions. Conidiophores of Piricauda, however, are not simple, but closely branched forming arched branches in the form of irregular semicircular loops, occasionally a zigzag structure is produced. Each arched hypha is a conidiophore bearing either conidia initials or a single conidium scar in the form of a pore

surrounded by a ring of darkened cell wall (Hughes 1960). Conidia of Piricauda are muriform with transverse and longitudinal septa, conidia of Porosubramaniana are 1-septate, exceptionally 2-septate.

Conidia of Porosubramaniana resemble conidia of Guedea Rambelli et Bartoli, however, the latter are blastic and borne on lateral, cylindrical denticles, conidiophores are erect, subcylindrical and mostly straight. Similar to the conidia of Guedea the conidia of Porosubramaniana have a terminal thin area of wall at the apical part which evidently is a germ pore.

Selenosporella-like synanamorph was found to belong to this species. The conidiogenous cells of synanamorph are borne on separate conidiophores, however, these conidiophores arise from the same basal hyphae as moniliform cicatrized fertile conidiophores of Porosubramaniana. Also conidia germinating at the papillate apex and forming a lageniform Selenosporella-like phialida were observed.

Up to now many species of different genera are known to produce "Selenosporella" synanamorph. This was noticed for example in Endophragmiella Sutton (Hughes 1979), Ceratosporium Schw. (Hughes 1964), Teratosperma Syd. et Syd. (Hughes 1952; Matsushima 1975) and in many others (see Carmichael *et al* 1980), in some records probably with a different conidium ontogeny similar to "Verticicladiella" or "Verticicladium" (Hughes 1979). In *Porosubramaniana moniliformis* the synanamorph is distinguished by a very long sporogenous part of the lageniform conidiogenous cell. This part is very minute and it seems that the origin of filiform conidia is polyphialidic on the sympodially prolongating sporogenous cells.

This interesting new species expects detailed microscopy studies, because by using only light microscopy it is not possible to explain some critical details referring to its conidium ontogeny as well as the conidium ontogeny of its synanamorph.

References

Carmichael J W, Kendrick W B, Conners I L and Sigler L 1980 *Genera of Hyphomycetes.* (Edmonton: The Univ. Alberta Press.) p. 168

Hughes S J 1952 Fungi from the Gold coast I; *Mycol. Pap.* **48** 1–91

Hughes S J 1960 Microfungi VI. Piricauda Bubák; *Can. J. Bot.* **38** 921–924

Hughes S J 1964 New Zealand Fungi 1. Ceratosporium Schw.; *NZ J. Bot.* **2** 305–309

Hughes S J 1979 Relocation of species of Endophragmia auct. with notes on relevant generic names; *NZ J. Bot.* **17** 139–188

Matsushima T 1975 *Icones microfungorum a Matsushima lectorum. Kobe* pp. 152–153

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 259–267.
© Printed in India.

Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete

NATHALIE BUFFIN and G L HENNEBERT

Laboratoire de Mycologie Systématique et Appliquée, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

Abstract. A new coprophilous Hyphomycete *Basifimbria spinosa* Buffin and Hennebert is described. The fungus is characterized by sympodial conidiophores producing two intergrading types of successive blastoconidia. The ones *Arthrobotrys*-like, unseptate, elliptical and smooth, born on successive denticulate swelling apices of the conidiophore; the others are *Basifimbria*-like, sphaerical and spinose, born on irregularly denticulate zig-zag conidiophores. The whole range of the conidiogenetical variation may occur on the same conidiophore. This pleoanamorphic fungus is, however, given one binomial only.

Keywords. Coprophilous Hyphomycete; *Basifimbria spinosa*; blastoconidia; conidiophores.

1. Introduction

The fungus, described here as a new species of *Basifimbria* Subramanian and Lodha, was found and isolated twice, as an *Arthrobotrys* species, on horse dung collected at Louvain-la-Neuve in 1976, after incubation in a moist chamber at room temperature for a few days.

When cultivated on malt yeast agar at 2% (MYA 2%) and corn meal agar (CMA) both isolates have successively developed two types of conidia. At first elliptical conidia of the *Arthrobotrys* type were developed on denticles at successive swollen ends of proliferating conidiophores. Later, the second kind of conidia, sphaerical and spinose, of the *Basifimbria* type appeared on the same proliferating conidiophores or on newly formed conidiophores. When those two types are developed on the same conidiophores, an intergrading form of conidia is observed, characterizing a progressively modified conidiogenesis. The last formed *Arthrobotrys* conidia become oboval and partially spinulose while the conidiogenous cell shows almost no swelling and less regularly grouped denticles.

Single conidium cultures have been prepared from *Arthrobotrys* type of conidia of each strain and have reproduced both *Arthrobotrys* and *Basifimbria* types of conidia. No germination has been obtained from the spinose sphaerical conidia of the *Basifimbria* type on the culture media used.

2. Description

Basifimbria spinosa Buffin and Hennebert n.sp.

Fungus anamorphicus ad Hyphomycetes pertinens.

Coloniae pallide cremeae usquead fulvae, tenues, pulverulentes, modice crescentes in maltoso geloso medio. Hyphae hyalinae, parietibus tenuibus, leves vel pauce punctatae, sparse septatae, ramosae, 1·5–3·7 μm latae. Conidiophora erecta, hyalina,

simplicia vel semel bisve furcata, recta vel geniculata, levia, parietibus tenuibus, 100–1075 μm longa, 5·2–7·5 usquead 3·7–4 μm lata, producentes successivas terminales conidiogenas cellulas et sympodialiter proliferantes. Conidiogenae cellulae arthrobotryoides, sympodiales, ferentes apicalites numerosa successiva conidia ad 1–1·5 μm latos denticulos in inflato apice, (25)50–80(125) μm longae, axialiter repetite proliferantes. Conidia holoblastica, unicellularia, ovata vel elliptica, apice rotundato, basiprominente, hyalina, tenuitunicata, levia, (13) 15–22(24,5) × 6–9(10,5) μm, liberata schizolytice. Conidiogenae cellulae, Basifimbriae typicae, sympodiales, ferentes apicaliter plures vel singula successiva conidia ad irregulariter dispositos interdum tarde septatos, 1–1, 5 μm latos denticulos vel ad curtos ramos 10–50 μm longa, axialiter vel saepe et invicem subaxialiter proliferantes. Conidia holoblastica, unicellularia, sphaerica, pallide lutea usque ochracea, crassotunicata, numerosis 1 μm long spinis ornata, (10,5) 13–16 μm in diametro, interdum obovata vel subglobosa, subtiliter vel parce spinulosa, tenui vel crasso pariete, 15–18,5 × 11–16 μm, liberata rhexolytice per fracturam denticuli vel schizolytice. Cellulae inflatae intercalares in hyphis, tenuitunicatae, 7–11 μm in diametro.

Typus lectus e stercore equino in Lovania Nova, Belgica, 24 Septembro 1976 a G L Hennebert in Herbaria MUCL 20173.

Colonies reaching 30 mm in diameter on CMA at 20°C after 5 days, submerged, translucid at the end covered by delicate powder-like conidiation, reaching 24 mm in diameter on MYA 2 % at 20°C (55 mm at 25°C; 15 mm at 30°C) after 5 days, with sparse aerial mycelium, light creamy to fulvous at sporulation. *Hyphae* hyaline, narrow, sinuose, septate at large intervals, 1·5–3·7 μm wide, thin-walled, sometimes slightly punctate. *Conidiophores* erect, rising from creeping narrow hyphae, simple or rarely branched, straight or geniculate, 100–1075 μm in length, 5·2–7·5 μm in diameter, the proliferation tapering to 3.7–4 μm in diameter, septate near the base and at regular intervals, smooth, thin-walled, producing successive terminal conidiogenous cells by axial or subaxial proliferations. *Conidiogenous cells* and *conidia* either of *Arthrobotrys* type or of *Basifimbria* type or intergrading from the former to the latter type on the same conidiophore. *Conidiogenous cell* of the *Arthrobotrys* type, sympodial, producing successive conidia acrogenously to subacrogenously in clusters at the increasingly swollen apex, proliferating axially with a septum above the original swelling and producing conidia on a denticulate swelling and so repeatedly at intervals of (25) 50–80 (125) μm. *Conidia* holoblastic, born successively on 1–1·5 μm wide denticles, at first apically then laterally on the increasingly swollen apex of the conidiogenous cell in cluster of up to 40 conidia, oboval to elliptical, rounded at the apex, (13–) 15–22 (24·5) × 6–9 (10·5) μm, smooth, hyaline, unseptate, thin-walled, seceding schizolytically, with a prominent 1–1·5 μm wide base bearing no frill, germinating apically or laterally by a long 2·5–3 μm wide germ tube. *Conidiogenous cell* of the *Basifimbria* type, sympodial, producing successive conidia acrogenously to subacrogenously, by small number or single at irregular intervals along the proliferating cell or on short branches, on denticles and without swelling, proliferating axially or often subaxially with sparse septa, giving a zig-zag pattern to the conidiophore. *Conidia* holoblastic, born successively on 1–1·5 μm wide denticles, apically or subapically irregularly grouped at the apex of the conidiogenous cell or on short lateral branches, globose (10·5) 13–16 μm in diameter, conspicuously spinose, spines 1 μ long, thick-walled, fulvous in mass, pale luteous to bright ochraceous under the microscope, with or without separating cell, seceding schizolytically or rhexolitically mostly leaving no conspicu-

ous frill at the conidium nor at the denticle. *Conidia* of intermediate type oboval or subglobose 15–18·5 × 11–16 μm thin to thick-walled, apically or partly and sparsely spinulose. *Swollen cells*, chlamydospores-like, born in hyphae often at bifurcate branching, intercalary, thin-walled, hyaline, smooth, 7–11 μm in diameter.

Material described: MUCL 20170 and MUCL 20173 (typus) on horse dung, Louvain-la-Neuve, Brabant, Belgique, collected and isolated by G L Hennebert on 24 September 1976, as dried culture in MUCL herbarium and living culture in MUCL Culture Collection (figures 1 and 2 from the type MUCL 20173).

3. Discussion

On horse dung incubated in moist chamber, the first habit shown by *Basifimbria spinosa* is that of an *Arthrobotrys* species. At the first stage of sporulation, indeed, on a culture like moistened horse dung, this new fungus species exhibits conidiophores similar to those of *Arthrobotrys* Corda (type species *Arthrobotrys superba* Corda). The conidiophores produce, in alternance to repeated vegetative proliferations, successively higher clusters of elliptical conidia developed in succession on denticles from the increasingly swollen apex.

Such conidiophores are of the "arthrobotryoid" type, one of the three types of conidiophores (arthrobotryoid, candelabroid, genicularoid) recognized by Jarowaya (1970) in *Arthrobotrys*. That type of conidiophore differs from the genicularoid type by the large number of conidia produced at intervals in opposition to the single conidium produced on the proliferating conidiophores of the other type, and from the candelabroid type by the short and compacted denticles forming nodes, instead of the long cylindrical branche-like denticles of the non-proliferating conidiophore of the latter type. In the arthrobotryoid type, Jarowaya (1970) distinguishes again two forms: one has apical proliferations producing a straight conidiophore as in *A. superba* Corda and *A. oligospora* Fresen., the other has subapical or lateral proliferations developing a geniculate conidiophore as in *A. arthrobotryoides* (Berl.) Lindau, *A. oligospora* Fresen., and *A. cladodes* Drechsler. In all these species, the development of the conidiophore is sympodial. The conidiophore does not proliferate during the conidiogenesis. The production of conidia is restricted to the apical zone that becomes nodose and eventually slightly swollen (*Arthrobotrys superba*) as a result of the conidiation. The proliferation is single, apical, axial or lateral.

Because of its straight proliferating conidiophores with denticulate swollen ampulliform nodes bearing elliptical conidia, *Basifimbria spinosa* might remind not only of *Arthrobotrys superba* but also of *Gonatobotrys simplex* Corda. Corda (1839) already showed the similar pattern of the two type species of *Arthrobotrys* and *Gonatobotrys*. But in *Arthrobotrys* the swelling occurs during the successive formation of conidia, while in *Gonatobotrys* the swelling of the ampulla occurs prior to the synchronous conidiation, as demonstrated by Drechsler (1950) in the type species *Gonatobotrys simplex* Corda and by Matsushima (1975) in *Gonatobotrys flava* Matsushima.

Conidia in *Arthrobotrys* are produced in succession at the apex of the conidiogenous cell but in an order that is not defined as yet in every species. The first conidium is born in the apical position, the second appears besides the first one and may or may not push it aside. The following conidia are formed apparently without any definite order

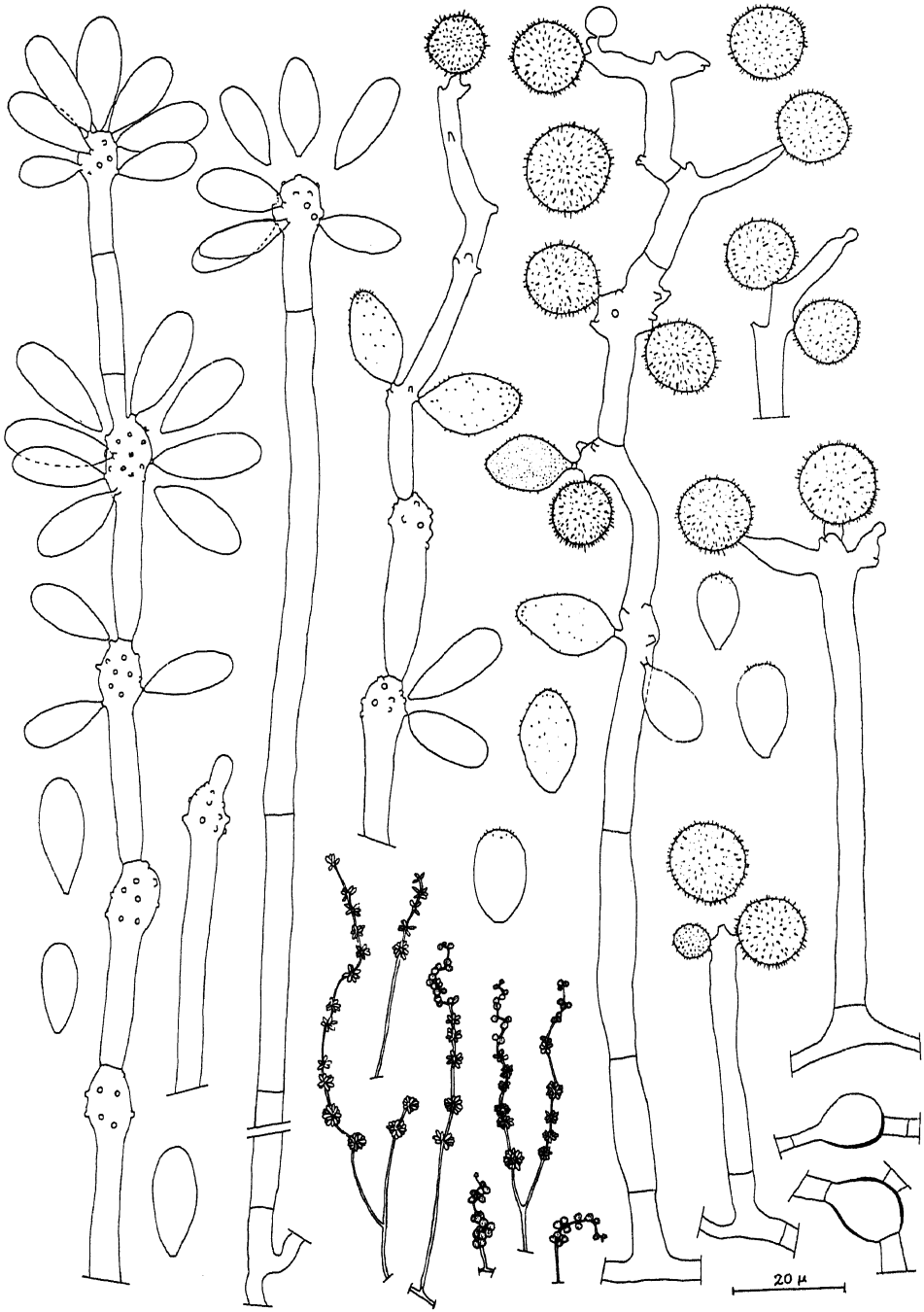


Figure 1. *Basifimbria spinosa* from type material MUCL 20173, on culture on MYA 2%.

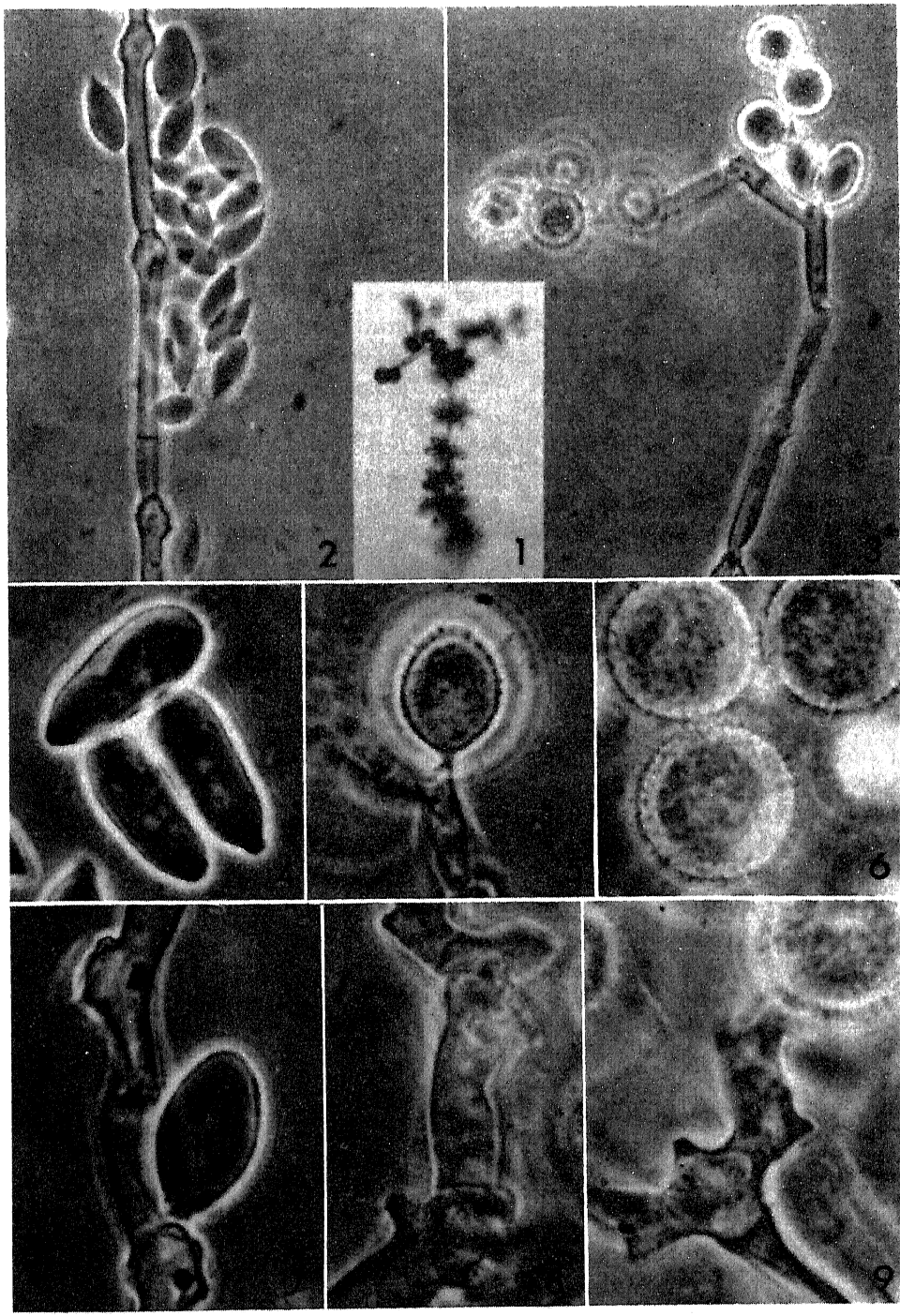


Figure 2. *Basifimbria spinosa* from type material MUCL 20173 in culture on MYA 2%; **1.** habit of an amphigenous conidiophore; **2, 4, 7.** parts of conidiophores and conidia of the *Arthrobotrys* type (600×, 1500×); **3, 6, 9.** parts of conidiophores and conidia of the *Basifimbria* type (600×, 1500×); **5, 8,** part of conidiophore and conidia of the intermediate type (1500×).

around the apex. In one species of *Arthrobotrys, A. amerospora*, however, conidia have been described by Schenk *et al* (1976) born in a basipital (retrogressive) succession, according to the interpretation of Barron (1979). In *Basifimbria spinosa*, we have not seen any youngest conidia developed at the base of the conidial cluster but well amongst the upper conidia of the cluster. We thus consider the conidiation in *Basifimbria spinosa* as sympodial and progressive.

Arthrobotrys Corda was described as having didymous conidia. But the generic concept has been recently amended through the successive inclusion of three *Arthrobotrys* species with amerous conidia: *A. anomala* Barron and Davidson, *A. amerospora* Schenk, Kendrick and Pramer and *A. botryospora* Barron. *A. anomala* is described with unseptate conidia while attached on the conidiophore but usually becoming one-septate before germination (Barron and Davidson 1972). We have not seen such a septation of conidia at the time of germination in *Basifimbria spinosa.* Further, the conidia of *Arthrobotrys anomala* are narrower (3–7 μm) and the conidiophores are smaller (20–80 μm). *A. amerospora* differs from our species by much larger conidia (15–31 × 10–20 μm) and the not swollen conidiogenous apex of the conidiophore. *A. botryospora* Barron (1979) has both septate and unseptate, 12–20 × 11–15 μm conidia that are wider than the elliptical conidia of *Basifimbria spinosa.* Schenk *et al* (1976), when describing *Arthrobotrys amerospora*, amended the generic concept. *Basifimbria spinosa* could thus also be assigned to *Arthrobotrys* Corda, as a fourth amerous conidial anamorph.

Basifimbria spinosa might be considered as not belonging to *Arthrobotrys* for its non-nematode-trapping hyphae. *Arthrobotrys* has been emphasized by Schenk *et al* (1976) Barron and Davidson (1972) and by Barron (1979) to be a genus of nematode-trapping fungi. For that reason Schenk *et al* transferred some *Dactylaria* species with one- or pluri-septate conidia and nematode-trapping organs to *Arthrobotrys. Basifimbria spinosa* does not produce any nematode-trapping organ, neither ring nor adhesive vesicle, when cultured in the presence or without nematodes from dung in various conditions known to be effective, on CMA (Comandon and de Fondbrune 1938; Barron 1977; Mankau 1980), on rhamnose and arabinose yeast agar (James and Nowakowski 1972) on cellulose paper and cellophane in water (Balan and Lechevalier 1972) and in peptone water (Jaskoski and Tortorello 1977). In the absence of nematodes, conidia of the *Arthrobotrys* type are dominant while in its presence conidia of the *Basifimbria* type prevail. *Arthrobotrys superba*, however, is said not predaceous (Jarowaya 1970).

Basifimbria spinosa also differs from the *Arthrobotrys* species by its plurality of conidial types which can be regarded as pleoanamorphy for it produces two kinds of conidia on distinctly differentiated conidiogenous cells. Such a feature is not recognized in *Arthrobotrys* species as yet.

Although some similarities with *Arthrobotrys* Corda, *Basifimbria spinosa* demonstrates characteristics of *Basifimbria* Subramanian and Lodha. *Basifimbria* was described with successive conidia formed acrogenously on sympodially developing sparsely branched and eventually subapically proliferating conidiophores.

The illustration of the type material published by Subramanian and Lodha (1968) clearly show these features. Subramanian and Lodha (1968) observed that "after the formation of the conidium a septum develops in the denticle separating the conidium from the conidiophore. The conidia are shed by a break of the denticle at any point

below the conidial septum leaving a part of the septate denticle on each conidium. The generic name *Basifimbria* is suggestive of the basal frill of the conidium". On the basis of a slide of the type culture RUBL 194 in DAOM Herbarium (DAOM 110030) Holubová-Jechová (1982) reassessed the genus and synonymized the coprophilous type species *Basifimbria aurea* Subramanian and Lodha with the lignicolous *Rhinotrichum subalutaceum* Peck as *Basifimbria subalutacea* (Peck) Holubová-Jechová. The two species indeed show many similarities. Holubová-Jechová also precises that the conidiogenesis is blastic, sympodial and follows a progressive sequence, the youngest conidium being found at the apex of the conidiogenous cell. She adds that the conidium is produced on a thin-walled denticle becoming lately septate at a short distance away from the conidium, delimiting a separating cell, and seceded rhexolytically, the wall of the separating cell being kept as a basal frill. The conidia result thus from a progressive succession and not a basipetal (retrogressive) succession as misinterpreted from the original illustration of the species by de Hoog and Hermanides-Nijhof (1977) and von Arx (1981) who synonymized *Basifimbria* with *Dicyma* Boulanger.

Basifimbria spinosa shows the same conidiogenesis as in *Basifimbria aurea* but evoluting from the one seen on the *Arthrobotrys*-like conidiophores. The vegetative proliferation and the conidial production on the conidiophore in strict alternance on the *Arthrobotrys*-like conidiophore become intermixed in such a way that the conidiophore produces smaller and irregular groups of conidia up to single conidium while proliferating axially or more often laterally. As a result the conidiophore becomes zig-zag and sparsely branched by proliferation. The schizolytical process of release of the *Arthrobotrys*-like conidia may change to a rhexolytical release with the development of a separating cell. Indeed, a cross septum led down in the denticle has been observed *in vitro* but not with consistency. The frill of the separating cell has not been seen on the conidium but can be hidden by the numerous 1·5 μm long spines ornamenting the conidial wall. It is a fact that after the release the denticle of the conidiogenous cell appears closed by a septum.

Furthermore *Basifimbria spinosa* produces sphaerical and ornamented conidia very similar to those of *B. aurea*. In that respect the two species differ only by conidial size.

It should be added that *Basifimbria spinosa*, like *B. aurea* was isolated from horse dung. The coprophilous character of *B. aurea* is also confirmed by the isolate that the junior author has obtained from horse dung recently.

Basifimbria spinosa is described with two but intergrading conidial morphogeneses. Should it be treated as a pleoanamorphic fungus composed of distinct synanamorphs, in the sense of the anatomical taxonomy and nomenclature?

The definition of a particular anamorph for nomenclatural purposes is not only a matter of deciding whether it is "an organ of asexual or somatic reproduction" (Weresub and Hennebert 1979) but a matter of taxonomic decision about what morphological or morphogenetical characters are delimiting one from another. The decision depends on the taxonomist.

It is now clear that in *Basifimbria spinosa* the taxonomist experienced in the scalpel art might be inclined to recognize a pleoanamorphic species composed of two synanamorphs pertaining to the form-genera *Arthrobotrys* and *Basifimbria* respectively and being denoted by two distinct binomials. So, we treated anatomically the Hyphomycete represented by the two synanamorphs *Cylindrodendrum album*

Bonorden and *Cylindrocarpon hydrophilum* Buffin and Hennebert (1984) in accordance with the International Code of Botanical Nomenclature. That was done for practical purposes of classification but willingly minimizing the intergradation between the two forms according to the culture conditions.

More than *Cylindrodendrum album, Basifimbria spinosa* is surely a disturbing case for the taxonomist. The single conidiophore is, at almost the same time, space and life conditions, amphigenous and must be dissected if one wants to distinguish, to separate and to name the synanamorphs. Even more, the intergradation of the characters from the base to the top of the conidiophore is such that it is troublesome to decide where to cut.

For that simple reason, a reason that justifies the application of a "botanical" system in the anamorphic fungi (one anamorphic fungus, one name) we have decided to denote our multiform conidial fungus by one name only in the anamorphic genus *Basifimbria*, avoiding to give it a second name in *Arthrobotrys*.

Similar investigations are carried out on the type species *Basifimbria subalutacea* (Peck) Holubová-Jechová (=*B. aurea* Subram. and Lodha) in order to demonstrate the taxonomic position of the genus.

Acknowledgement

This study is acknowledged as a result of the MUCL cataloguing financed by the Belgian Science Policy Programming Services in the framework of the national coordinated collection of microorganisms (SPPS Bio 1.A.1.2. 1983–1987).

References

Balan J and Lechevalier A 1972 The predaceous fungus, *Arthrobotrys dactyloides*, induction of trep formation; *Mycologia* **64** 919–922

Barron G L 1977 The nematode-destroying fungi. *Topics in Mycobiology n°* 1; Can. Biol. Publication Ltd. 140p

Barron G L 1979 Nematophagous fungi: a new *Arthrobotrys* with non-septate conidia; *Can. J. Bot.* **57** 1371–1373

Barron G L and Davidson J G N 1972 Nematophagous Hyphomycetes: *Arthrobotrys anomala* sp. nov; *Can. J. Bot.* **50** 1773–1774

Buffin N and Hennebert G L 1984 *Cylindrodendrum album* Bonorden, a pleoanamorphic semiaquatic Hyphomycete; *Mycotaxon* **19** 323–341

Comandon J and de Fondbrune P 1938 Recherches expérimentales sur les champignons prédateurs des nématodes du sol. Les pièges garotteurs; *C. R. Soc. Biol. Paris* **129** 620–625

Corda A J C 1839 *Pracht Flora der Europaeischer Schimmelbildungen* (Leipzig and Dresden) 55 p. 25 pl

De Hoog G S and Hermanides-Nijhof E J 1977 Survey of the black yeasts and allied fungi. *Studies in mycology* **15** 178–222

Drechsler C 1950 Several species of *Dactylella* and *Dactylaria* that capture freeliving nematodes; *Mycologia* **42** 1–79

Holubová-Jechová V 1982 Some notes on *Basifimbria aurea*; *Mycotaxon* **14** 140–144

James A W and Nowakowski R J 1968 The effect of carbon sources on trap formation by *Arthrobotrys conoides*; *Can. J. Microbiol.* **14** 1260–1261

Jarowaya N 1970 The genus *Arthrobotrys*; *Acta Mycol.* **6** 337–406

Jaskoski B J and Tortorello M L 1976–1977 Namatode age and trap formation in *Arthrobotrys conoides*; *Trans. Ill. State Acad. Sci.* **69** 255

Mankau R 1980 Biocontrol fungi as nematode control agents; *J. Nematol.* **12** 244–252

Matsushima T 1975 Icones microfungorum a Matsushima lectorum. Kobe. 210p. 415 pl

Schenk S, Kendrick W B and Pramer D 1976 A new nematode-trapping Hyphomycete and a re-evaluation of *Dactylaria* and *Arthrobotrys*; *Can. J. Bot.* **55** 977–985

Subramanian C V and Lodha B C 1968 Two interesting coprophilous fungi from India; *Curr. Sci.* **37** 245-248

von Arx J A 1982 The genus *Dicyma*, its synonyms and related fungi; *Proc. Ned. Akad. Wet.* **85** 21–28

Weresub L and Hennebert G L 1979 Anamorph and teleomorph: terms for organs of reproduction rather than karryological phases; *Mycotaxon* **8** 181–186

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 269–272.
© Printed in India.

Two new hyphomycetes from Ethiopia

D J BHAT

Department of Biology, Faculty of Natural Sciences, Asmara University, P.O. Box 1220, Asmara, Ethiopia

Abstract. Two dematiaceous hyphomycetes, *Spadicoides subramanianii* sp. nov. and *Dwayabeeja aethiopica* sp. nov., collected on dead twigs from Ethiopia are described and illustrated.

Keywords. Fungal taxonomy; hyphomycetes.

It is an honour to have been invited to contribute to this volume celebrating the 60th birthday of Prof. C V Subramanian. As one of his former students who spent seven years at the University Botany Laboratory, Madras, India, I acknowledge that Prof. Subramanian was not only responsible for stimulating my interest in hyphomycetes but subsequently by both teaching and his own example virtually taught me everything that I now know.

***Spadicoides subramanianii* sp. nov.**
(Etym. in honour of Prof. C V Subramanian)

Coloniae effusae, atrobrunneae, velutinae. Conidiophora mononematosa, ramosa, erecta, recta vel flexuosa, laevia, cylindrica, atrobrunnea, 160–215 μm longa, 6·5–8·5 μm lata, septata, ramis ad latus 0–4 septatis usque ad 60 μm longis, statim infra septa orientibus, in parte superiore plerumque fertilia. Cellulae conidiogenae polytreticae, in conidiophoris incorporatae, usque ad 30 poris minutis temere distributis, post secessio conidiorum visibilibus. Conidia solitaria, sicca, primo ellipsoidea vel oblonga, dein doliiformia, laevia, 2-euseptata, septis crassis, utrinque extremis obtusa, medio vel atrobrunnea, 6·4–8·5 μm lata; cella mediana 8–12 μm longa, 8 μm lata; cellis extremis 4 μm longis, saepe collapsis.

Holotypus in ramulis delapsis ignotis, 26 km Mizan Teferi ad Tepi, Kaffa, Ethiopia, D J Bhat, Jan. 27, 1984, Herb. NMAU 072.

Colonies effuse, dark brown, velvety. Conidiophores mononematous, branched, erect, straight or flexuous, smooth, cylindrical, dark brown, 160–215 μm long, 6·5–8·5 μm wide, septate, with 0–4 septate side branches up to 60 μm long arising immediately below the septa, fertile in the upper part. Conidiogenous cells polytretic, integrated, with up to 30 or more randomly distributed pores visible after secession of conidia. Conidia solitary, dry, initially ellipsoidal to oblong, finally doliiform, smooth, 2-euseptate, with thick septa, rounded at both ends, mid- to dark brown, 6·4–8·5 μm wide; middle cell 8–12 μm long, 8 μm wide; end cells 4 μm long, frequently collapsing.

Holotype on fallen twigs, 26 km from Mizan Teferi to Tepi, Kaffa, Ethiopia, collected by D J Bhat, Jan. 27, 1984, Herb. NMAU 072.

In its general morphology, the fungus possesses characters of two closely related genera, *viz. Spadicoides* Hughes and *Diplococcium* Grove (Ellis 1971). The type species

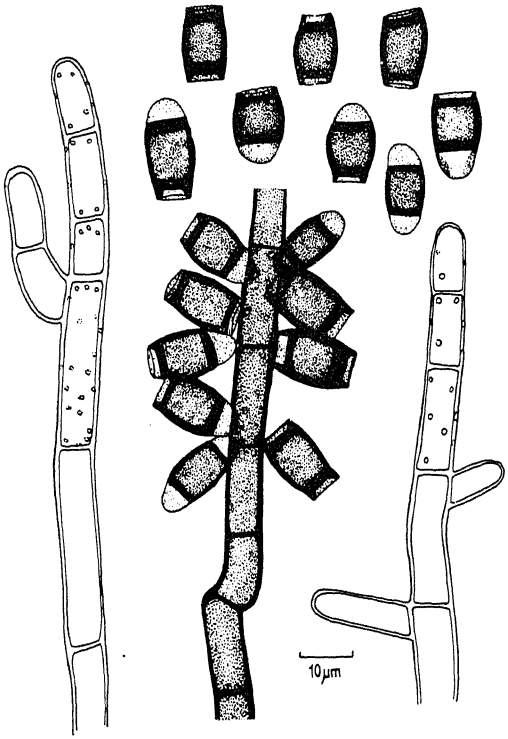


Figure 1. *Spadicoides subramanianii*—conidiophores and conidia.

of *Spadicoides* Hughes, *S. bina* (Corda) Hughes, is characterised by unbranched conidiophores with integrated conidiogenous cells producing solitary conidia. *Diplococcium spicatum* Grove, type of *Diplococcium* Grove, has much branched conidiophores producing catenate conidia on integrated conidiogenous cells. Wang and Sutton (1982) questioned the separation of *Spadicoides* and *Diplococcium* on catenation of conidia. *Spadicoides subramanianii* provides another example illustrating the unsatisfactory way in which generic concepts in these two genera are used, because although the conidia are solitary, the conidiophores do branch. The solitary conidia which on secession become doliiform are very typical of this fungus. It is unlike any hitherto known species of *Spadicoides* or *Diplococcium*.

***Dwayabeeja aethiopica* sp. nov.**
(Etym. after Ethiopia)

Coloniae effusae, atre, pulveraceae. Conidiophora parva, 3–4 septata, nonramosa, pallide brunnea, verruculosa, 2·5–8 μm lata. Cellulae conidiogenae polyblasticae, primum subglobosae, dein cupulatae, verruculosae, in parte inferiore atrobrunneae, parietibus crassis, in parte superiore pallide brunneae et collapsae, 6·5–8 μm diam., conidiis brevibus vel longis producentes. Conidia solitaria, sicca (interdum brevia in apicem conidium singulum additum producentia), 1–3 in parte superiore cellularum conidiogenarum formata, simplicia, verrucosa, phragmospora; conidia-brevia elon-

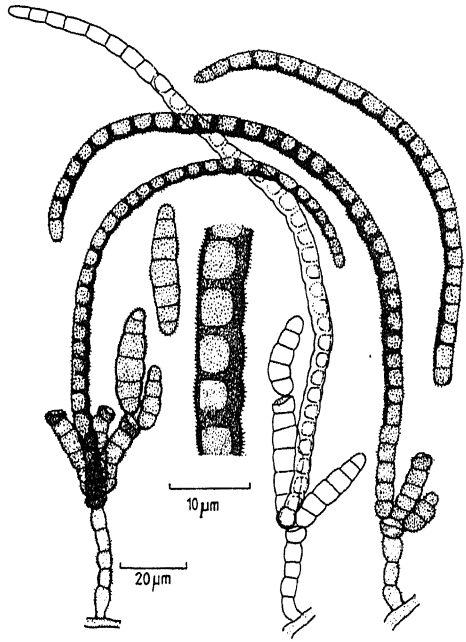


Figure 2. *Dwayabeeja aethiopica*—conidiophores and conidia.

gata vel fusiformia, recta vel curvata, 2–8 septata, ad septis constricta, medio vel atrobrunnea, utrinque extremis pallide brunnea, in mediano latissima, utrinque extremis obtusa, 20–40 µm longa, 6·5–8 µm lata; conidia-longa flagelliformia, constanter curvata, usque ad 39 cellulis composita, brunnea, apicem versus pallidiora, parietibus periclinalibus inaequaliter crassis, versus latum incrassatum curvatura et in latum incrassatum septis constrictis, usque ad 230 µm longa, 5–7 µm lata.

Holotypus in ramulis delapsis ignotis, 21 km Bonga ad Felegesalam, Kaffa, Ethiopia, D J Bhat, Jan. 25, 1984, Herb. NMAU 037.

Colonies black, powdery, effuse. Conidiophores small, 3–4 septate, unbranched, pale brown, verruculose, 2·5–8 µm wide. Conidiogenous cells polyblastic, at first subglobose, later cupulate, verruculose, with the lower part dark brown, thick-walled, the upper part pale brown and soon collapsing, 6·5–8 µm diam, producing both short and long conidia. Conidia solitary, occasionally short conidia produce an additional one at the apex, dry, arising singly or in twos or more from the upper part of the conidiogenous cells, simple, verruculose, multiseptate; short conidia elongate-fusiform, straight or curved, 2–8 septate, constricted at the septa, mid to dark brown, paler at both ends, broadest in the middle, with smoothly rounded end cells, 20–40 µm long, 6·5–8 µm wide; long conidia whip-like, consistently curved, composed of up to 39 cells, brown, paler towards the apex, with unequally thickened periclinal walls, the curvature towards the thickened side, constricted at the septa always on curved side, up to 230 µm long, 5–7 µm wide.

Holotype on fallen twigs, 21 km from Bonga to Felegesalam, Kaffa, Ethiopia, collected by D J Bhat, Jan. 25, 1984, Herb. NMAU 037.

The genus *Dwayabeeja* Subramanian, typified by *D. sundara* Subramanian, is characterised by toruloid conidiophores producing two kinds of conidia, one short and the other long, on the same conidiogenous cell (Ellis 1971). *D. sundara* is the only species so far known in the genus. The unevenly thickened nature of the walls of long conidia, their curvature on the thickened side and the overall conidial dimensions of *D. aethiopica* are quite different from those of *D. sundara*.

Acknowledgements

I am grateful to Dr B C Sutton of Commonwealth Mycological Institute, Kew, England, for assistance in preparing this manuscript. I am thankful to the Royal Society, London, for the award of a Developing Country Fellowship during the tenure of which at the Commonwealth Mycological Institute, Kew, this paper was written. Constant encouragement from Dr Tewolde Berhan Gebregziabier, President, and Ato Zere Izaz, Academic Vice President, Asmara University, is duly acknowledged.

References

Ellis M B 1971 *Dematiaceous hyphomycetes* (Kew, Surrey, England: Commonwealth Mycological Institute) 608 pp.

Wang C J K and Sutton B C 1982 New and rare lignicolous hyphomycetes; *Mycologia* **74** 489–500

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 273–280.
© Printed in India.

Pigment localization in Dematiaceous Hyphomycetes and the segregation of *Pseudogliomastix* gen. nov. from *Acremonium*

W GAMS and T BOEKHOUT

Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, Netherlands

Abstract. Conidia of *Acremonium polychromum* and of the anamorph of *Wallrothiella subiculosa* were studied with TEM. The conidia of *A. polychromum* have a very thick, fibrous, electron-dense outer layer, which finally becomes coarsely warted. In *W. subiculosa* this layer remains thin and more compact and becomes minutely roughened. Because of these differences and the regular pigmentation of the vegetative hyphae and phialides, a new genus, *Pseudogliomastix*, is described for this anamorph. The differences between pigmented and hyaline conidia among species of *Acremonium* sect. *Gliomastix* do not seem to be so fundamental as to preclude their inclusion in one genus, *Acremonium*.

Keywords. Dematiaceous Hyphomycetes; ultrastructure; pigmentation; taxonomy; *Acremonium; Gliomastix; Pseudogliomastix; Wallrothiella.*

1. Introduction

The delimitation of the genera *Acremonium* Link: Fr., *Gliomastix* Guéguen, and *Sagrahamala* Subram. based on conidial pigmentation and chain formation has been a matter of dispute. The esteemed Prof. Dr C V Subramanian, whose contributions to mycology are honoured with this issue, has been a driving force in this discussion (Subramanian 1973; Subramanian and Pushkaran 1976). Features used to distinguish between false and true chains were dealt with in a previous paper (Gams 1978) and here we shall confine ourselves to pigmentation. We hypothesize that the distribution of pigments within the conidial wall may have more taxonomic value than hitherto recognized.

It is now generally accepted that within the Hyphomycetes the distinction between the form-families Dematiaceae with dark structures and Mucedinaceae with only hyaline structures is no longer tenable. Consequently Ellis (1971, 1976) did not use these terms as names of families but only in adjectival form as convenient descriptive terms. To reach a workable classification, pigment distribution must be more accurately defined, firstly, along different parts of the thallus (particularly conidiophores and parts thereof and conidia) and, secondly, in different wall layers of the pigmented elements.

In analogy with *Stachybotrys* Corda (see Jong and Davis 1976), Gams (1971) included species with both hyaline and pigmented conidia in one genus, *Acremonium*. Pairs of very similar species were repeatedly observed, which differed only in the presence or absence of conidial pigmentation. The species formerly classified in *Gliomastix* were classified in a separate section, *Gliomastix*; this section, however, was not delimited because of conidial pigmentation, but mainly because of the presence of thickened, chondroid hyphae, which render the colony very tough. Objections against this procedure were summarized by Hammill (1981). Gams (1971) was not entirely consistent in applying the criterion of chondroid hyphae and included the *A. striatisporum* series with pigmented conidia which lacked thickened hyphae; this

series was subsequently (Gams 1978) transferred to a separate genus, *Sagenomella*, because of "connected" conidial chains (better "chains with connectives", according to Minter *et al* 1983). The species *A. inflatum* and *A. atrogriseum* also lack chondroid hyphae and should be classified elsewhere. On the other hand, the anamorph of *Wallrothiella subiculosa* Höhnel, known as *Gliomastix protea* (Sacc.) Verona and Castella, was deliberately excluded from *Acremonium* because its vegetative hyphae are often pigmented and the pigmentation of the smooth yellow-brown conidia differs strongly from the olivaceous-brown encrusted conidia of other *Gliomastix* species. *Wallrothiella* should therefore not be considered as a teleomorph of *Gliomastix* or even *Acremonium*, as was done in the symposium "The whole fungus" (edited by Kendrick 1979) by Subramanian (p. 138) and Samuels and Rossman (p. 172), and also by Carmichael *et al* (1980). A separate genus is required for this fungus in order to preclude any misunderstanding. Whether the pigmentation develops in a similar way amongst species of *Gliomastix* and whether this is fundamentally different from the development of pigmentation in conidia of *Wallrothiella subiculosa*, is the topic of this study.

2. Material and methods

The following species and isolates were examined (in two-week-old cultures on 2% MEA):

Acremonium polychromum (van Beyma) W. Gams, CBS 511·63, conidia almost smooth, darkly pigmented; phialides hyaline, sometimes pigmented at the tip.

Wallrothiella subiculosa Höhnel, CBS 389.73, conidia smooth, gradually becoming yellow-brown, as dark as the phialides.

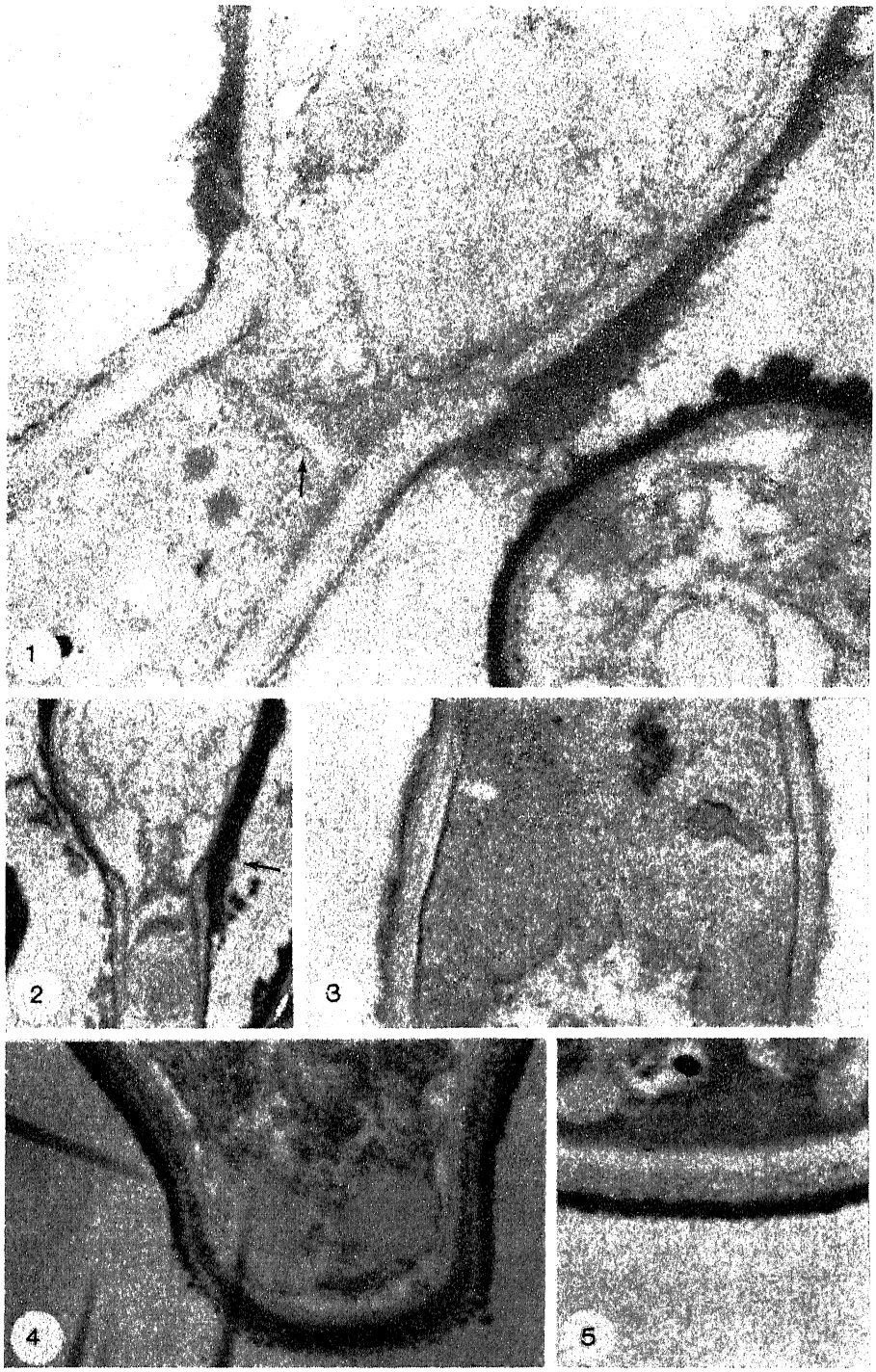
Bleaching of pigment: Fragments of the agar cultures were transferred into a concentrated solution of commercial sodium hypochlorite (containing 10% active Cl) or in 5% KOH.

Transmission electron microscopy: The material was prefixed with 2% glutaraldehyde and 5% dimethylsulphoxide in 0·05 M phosphate buffer (pH 7·2), for about 20 min. A subsequent treatment with snailgut juice (0·5% v/v in a buffer containing 30 mM EDTA, 30 mM $MgSO_4$ and 50 mM mercaptoethanol) for about 30 min at 30°C was followed by fixation in 3% glutaraldehyde in 0·05 M phosphate buffer (pH 7·2) for 1 hr. The material was then washed several times in buffer and postfixed in 1% OsO_4 in 0·05 M phosphate buffer (pH 7·2) for 1 hr. After fixation the material was rinsed in 50% (v/v) acetone for 30 min and blockstained in 1·2% uranyl acetate in 70% (v/v) acetone for 30 min. This was followed by dehydration in a graded acetone series with a final addition of some drops of 2,2-dimethoxypropane (Linnemans *et al* 1977) and embedding in araldite. The sections were stained in 4% aqueous uranyl acetate for 5 min, followed by lead citrate for 1 min.

3. Results

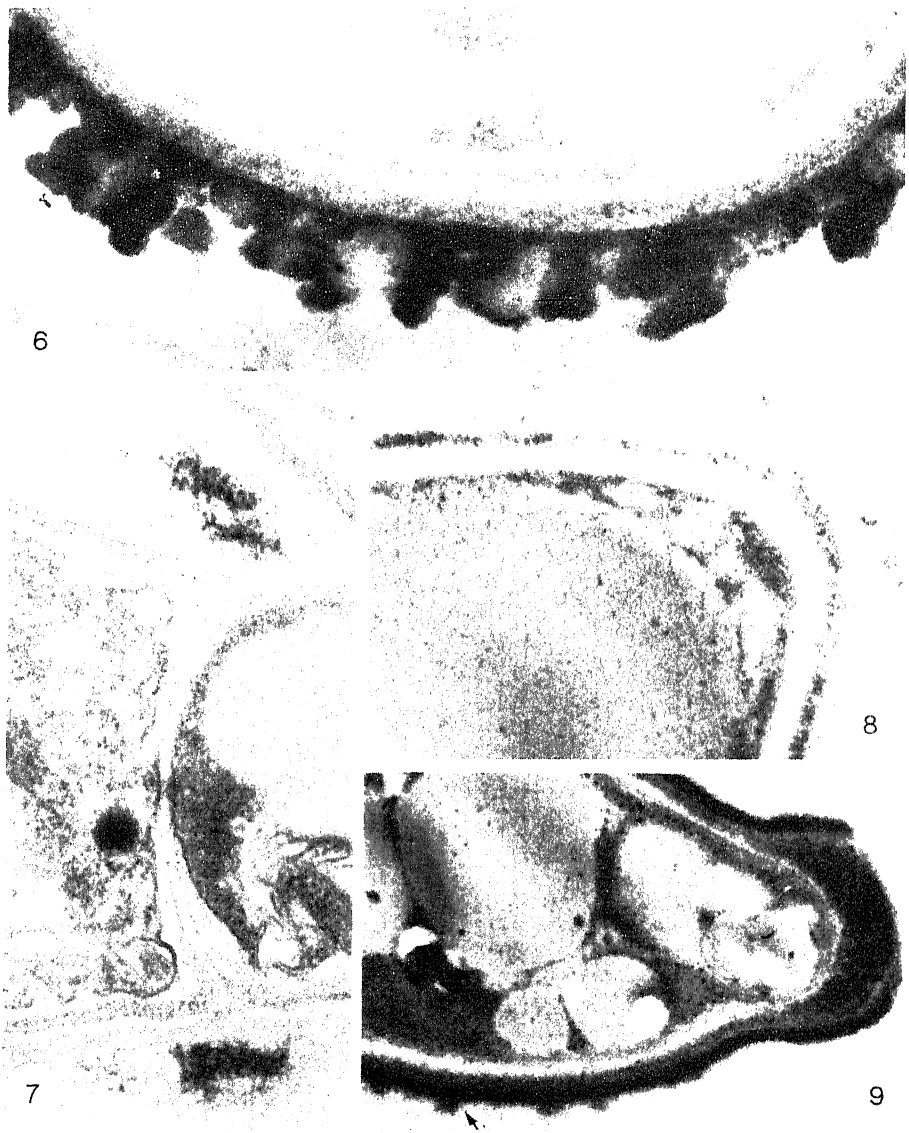
3.1 *Acremonium polychromum*

The hyphal wall is layered and consists of approximately 4 layers, which differ slightly in their electron opacity (figure 3) and is covered by a thin electron-dense sheath. Like



Figures 1–5. *Acremonium polychromum*. **1, 2.** Apex of phialide with conidium (× 36000 and × 8400). **3.** Layered hyphal wall. **4.** Conidial scar of young conidium. **5.** Wall of young conidium (3–5 × 36000).

other hyphae, the phialides contain an inner layer of low electron density; this layer gradually thickens towards the apex and forms a periclinal annular thickening characteristic of phialidic conidiogenesis (figures 1, 2). The conidia are extruded from the phialide opening. The walls of the young conidia are inconspicuously layered and of medium electron density. The outermost, electron-dense fibrillar sheath seems to be continuous with that covering the phialide (figures 1, 2 arrow). A transverse septum is



Figures 6–9 (× 36000) **6.** *Acremonium polychromum*, wall of mature conidium. **7–9.** Anamorph of *Wallrothiella subiculosa*. **7.** Hyphal septum. **8.** Wall of young conidium. **9.** Wall of mature conidium.

formed inside the phialide, just below its apex (figure 1 arrow), finally separating the conidium and the phialide.

Young conidia appear nearly smooth. The walls of the young conidia are of medium electron density; an outer layer containing small electron-dense spots is covered by a thin electron-dense sheath (figures 4, 5). Mature conidia have essentially the same layers, but the electron-dense sheath has a homogeneous or fibrillar texture and has increased considerably in thickness. Finally the surface becomes warted due to the deposition of lumps of pigment. Just beneath this layer, a zone with small, dispersed, electron-dense spots occurs in the less electron-dense layer of the conidial wall (figure 6). The pigmented sheath of the conidium is less developed near the conidial scar (figure 4).

Bleaching experiments resulted in sudden disappearance of pigment in NaOCl but no reaction to KOH.

Probably the very thick warty pigmented sheath is a result of secondary excretion of pigment through the inner layers of the cell wall. This is schematically presented in figure 10A.

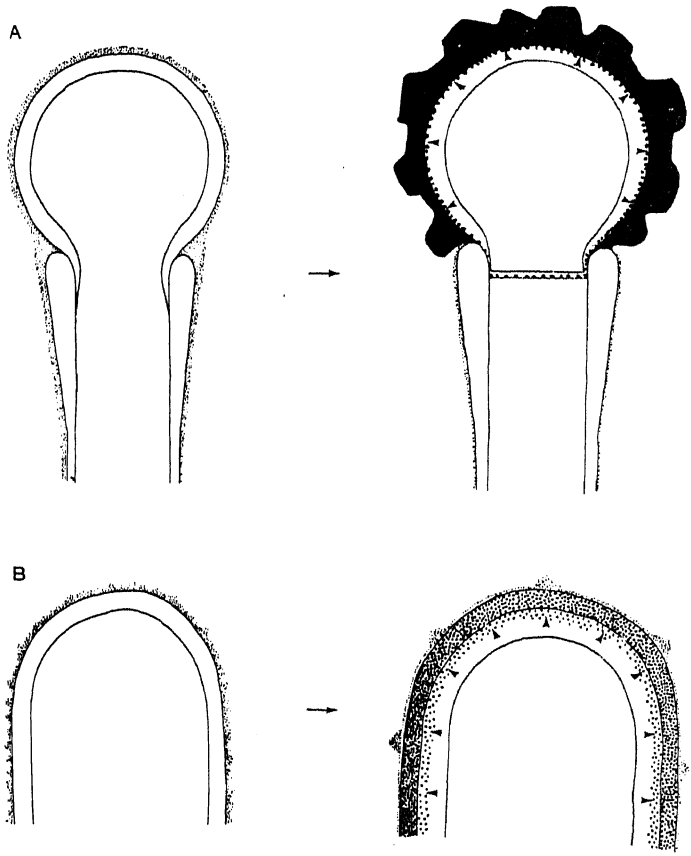


Figure 10. Diagrammatic interpretation of the development of A. the conidial wall in *A. polychromum* and **B.** the anamorph of *W. subiculosa*. Arrow heads indicate the supposed deposition of pigment.

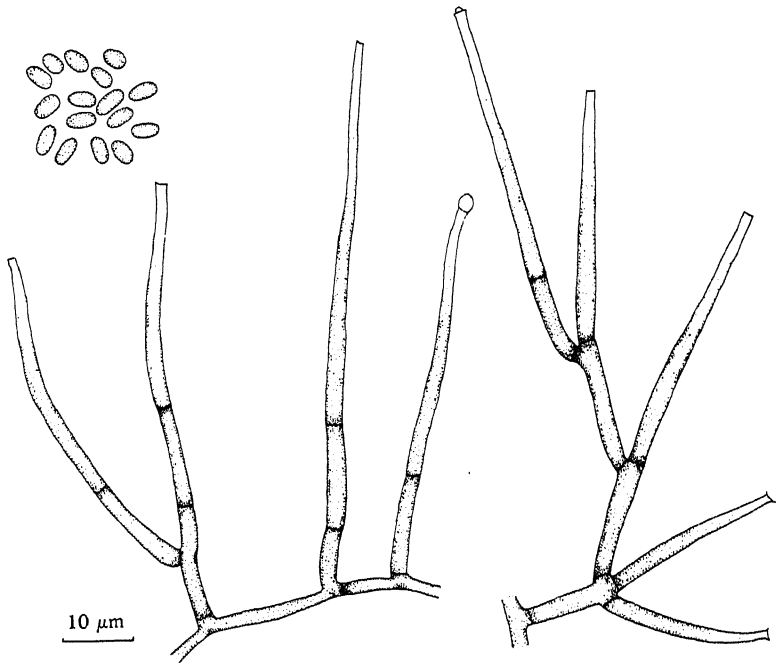


Figure 11. *Pseudogliomastix protea*, the anamorph of *Wallrothiella subiculosa*, herb. IMI 31229a and 56093a.

3.2 *Wallrothiella subiculosa*

The hyphae are covered by a thick capsule (figure 7). Electron-dense lumps occur in the outer fibrillar layer adjacent to the septa, thus forming an annular zone. The proper cell wall is layered and of medium electron density. Two thin outer zones occur which are slightly more electron-dense.

The wall of presumably young conidia (figure 8) has a relatively thin, electron-dense floccose outer layer and a rather thick inner layer of low electron density. In mature conidia the outermost electron-dense sheath remains thin, if compared with *A. polychromum*, and only small warts appear on the surface of the compact outer layer (figure 9 arrow). Just beneath this layer a zone of small electron-dense spots occurs in the less electron-dense inner layer, probably also indicating an excretion of pigment through the inner layer towards the outer layers of the wall. This is schematically presented in figure 10B.

The conidia react more tardily to bleaching with NaOCl, which takes more than 10 sec, KOH has no noteworthy effect. This again indicates a deeper localization of the pigment.

4. Discussion

Reisinger and Guédenet (1968), Reisinger (1969), Reisinger and Bonaly (1972), and Cole (1973) showed that in pigmented hyphae and conidia amorphous material or

granules are often deposited at a later stage in and on the outermost wall layer (which they called layer A), followed by more or less pigment accumulation within the underlying layer B. This pigment localization becomes clearly visible in ultrathin sections under TEM after trypsin or HCl treatment of the walls (Reisinger and Bonaly 1972). This situation is particularly pronounced even at the light-microscopic level in the chlamydospores of *Humicola* (Griffiths 1974). In *Stachybotrys* as well as in *Memnoniella*, Campbell (1972, 1975) showed by TEM that conidia become pigmented in this way. This kind of secondary pigment deposition may explain the unusual feature that closely related fungi can either have hyaline or darkly pigmented conidia.

The mechanisms of formation and deposition of pigment have not been elucidated in this study. It seems possible, however, that the pigment of the conidia of *A. polychromum* originates initially partly from the electron-dense sheath surrounding the hyphae and phialides, but the majority seems to be a secondary deposition from the cytoplasm onto the conidial wall surface. Probably a similar mechanism also operates in the conidia of *W. subiculosa*, although most of the pigment becomes more homogeneously localized within the wall layers.

It can be concluded that the differences between *W. subiculosa* and *A. polychromum* at the submicroscopic level reflect those observed at the light-microscopic level. The conidia of the latter have a very thick electron-dense outer layer, which finally becomes roughened by lumps of pigmented material, whereas in the conidia of *W. subiculosa* this layer remains thinner and more compact and the warts are smaller. As *A. polychromum* has less roughened conidia than most of the other species of the section with pigmented conidia, a generalization of our observations on these species seems to be justified. However, a more detailed study involving several species is required.

These findings confirm the previous conclusion (Gams 1971) that the anamorph of *Wallrothiella subiculosa* differs fundamentally from other pigmented species of *Acremonium* sect. *Gliomastix*. Therefore we describe a new genus for it.

Pseudogliomastix W. Gams, gen. nov. (figure 11)

Genus Hyphomycetum, *Acremonii* simile, sed hyphis vegetativis et conidiophoris pigmentatis differens. Conidia dilute brunnea in tota pariete colorata.

Species typica *Pseudogliomastix protea* (Sacc.) W. Gams, comb. nov. (Basionym: *Torula protea* Sacc., Michelia **2** 292. 1881, and Fungi Ital. delin. figure 951 1881), syn. *Gliomastix protea* (Sacc.) Verona and Castella, Annali Fac. Agr. Univ. Pisa, N.S. **5** 383 1942.

Teleomorph *Wallrothiella subiculosa* Höhnel, Sber. Akad. Wiss. Wien **121** 381. 1912 (Fragm. Mykol. 763).

Type material of anamorph on *Arundo donax*, Padova, Italy, Mycotheca veneta 1591, in PAD.

Full descriptions in Hughes and Dickinson (1968), Dickinson (1968) and Gams (1971).

Acknowledgement

The authors are indebted to Mr K A Seifert for corrections of the English text.

References

Campbell R 1972 Ultrastructure of conidium ontogeny in the deuteromycete fungus *Stachybotrys atra* Corda; *New Phytol.* **71** 1143–1149

Campbell R 1975 The ultrastructure of the formation of chains of conidia in *Memnoniella echinata; Mycologia* **67** 760–769

Carmichael J W, Kendrick W B, Conners I L and Sigler L 1980 *Genera of Hyphomycetes.* (Edmonton: Univ. Alberta Press) 386 pp.

Cole G J 1973 Ultrastructure of conidiogenesis in *Drechslera sorokiniana; Can. J. Bot.* **51** 629–638

Dickinson C H 1968 *Gliomastix* Guéguen; *Mycol. Pap.* **115** 24 pp.

Ellis M B 1971 *Dematiaceous Hyphomycetes* (Kew: Commonwealth Mycological Institute) 608 pp.

Ellis M B 1976 *More Dematiaceous Hyphomycetes* (Kew: Commonwealth Mycological Institute) 507 pp.

Gams W 1971 *Cephalosporium*-artige Schimmelpilze (Hyphomycetes). G. Fischer, Stuttgart. 262 pp.

Gams W 1978 Connected and disconnected chains of phialoconidia and *Sagenomella* gen. nov. segregated from *Acremonium; Persoonia* **10** 97–112

Griffiths D A 1974 Development and structure of the aleuriospores of *Humicola grisea* Traaen; *Can. J. Microbiol.* **20** 55–58

Hammill T M 1981 On *Gliomastix murorum* and *G. felina; Mycologia* **73** 229–237

Hughes S J and Dickinson C H 1968 New Zealand fungi XI. Gliomastix Guéguen; *N. Z. J. Bot.* **6** 106–114

Jong S C and Davis E E 1976 Contributions to the knowledge of *Stachybotrys* and *Memnoniella* in culture; *Mycotaxon* **3** 409–485

Kendrick B (ed.) 1979 *The whole fungus* (Ottawa: National Museum of Natural Sciences) 793 pp.

Linnemans W A M, Boer P and Elbers P F 1977 Localization of acid phosphatase in *Saccharomyces cerevisiae*: a clue to cell wall formation; *J. Bacteriol.* **31** 638–644

Minter D W, Kirk P M and Sutton B C 1983 Thallic phialides; *Trans. Br. mycol. Soc.* **80** 39–66

Reisinger O 1969 Morphologie ultrastructurale et critères taxinomiques chez les Deutéromycètes. II. L'appareil sporifère chez *Dendryphiella vinosa* (Berk. et Curt.) Reisinger; *Rev. Mycol.* **34** 109–121

Reisinger O and Guédenet J C 1968 Morphologie ultrastructurale et critères taxinomiques chez les Deutéromycètes. I. Les parois sporales chez *Dendryphiella vinosa* (Berk. et Curt.) Reisinger; *Bull. trimest. Soc. mycol. Fr.* **84** 19–26

Reisinger O and Bonaly R 1972 Etudes des modifications des parois de *Dendryphiella vinosa* (Berk. et Curt.) Reisinger après traitements chimiques et enzymatique; *C. R. hebd. Séances Acad. Sci. Paris* **274** 50–53

Subramanian C V 1973 Reflections on the conidium; *J. Indian bot. Soc.* **52** 3–16

Subramanian C V and Pushkaran M 1976 Conidium ontogeny in some monophialidic Hyphomycetes; *Kavaka* **3** 77–99 ("1975").

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 281–308.
© Printed in India.

A re-appraisal of the relationships between *Arthrinium* and other hyphomycetes

DAVID WILLIAM MINTER
Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, TW9 3AF, UK

Abstract. Conidial development in *Arthrinium* and similar genera with basauxic conidiophores is reviewed and compared with that of *Nigrospora*. A hypothesis is erected to explain the nature of the basauxic conidiophore type of development. Predictions are made from the hypothesis, and are tested using species of *Chalara, Mammaria, Wardomyces* and selected other hyphomycete genera as examples. It is concluded that, to classify these hyphomycetes, a system is required which would completely cross the boundaries of the traditional classifications of Saccardo and Hughes.

Keywords. Classification; conidiogenesis; hyphomycetes; *Arthrinium*

1. Introduction

Arthrinium Kunze is one of a small cluster of hyphomycete genera with a curious combination of developmental characteristics which makes them appear strikingly different from any other imperfect fungi: they have so-called basauxic conidiophores, which arise from structures, apparently unique to this group, called conidiophore mother cells (Hughes 1953). The fungi are comparatively well known. They were discussed by Mason (1933), Ellis *et al* (1951) and Cooke (1954). Many species have been beautifully illustrated by Ellis (1971, 1976) and Matsushima (1975), and a useful account of their development, with some ultrastructural evidence, has been provided by Cole and Samson (1979). On the basis of their curious features, Hughes (1953) in his seminal work on hyphomycete classification treated these genera as belonging in a group all of their own and Tubaki (1958) in a similarly influential paper concurred.

In recent years few mycologists have seriously questioned this viewpoint, a notable exception being Subramanian (1979), who pointed out the remarkable developmental similarities between certain types of phialide and the so-called conidiophore mother cells of these fungi, thus reviving an old view (Ellis *et al* 1951; Cooke 1954) which had lost favour with other mycologists. Against this background a considerable re-appraisal of our understanding of deuteromycete development is now taking place (Minter *et al* 1982, 1983a, b; Minter 1984), and given the nature of the present volume, celebrating the contributions to mycology by that eminent student of the hyphomycetes, Prof. C V Subramanian, it seems highly appropriate to reconsider these beautiful fungi.

The aim of the present paper is therefore to try to explain the enigmatic development found in this group. This work will not attempt to revise species concepts nor will it provide a formal taxonomic revision of this group. It will simply address the question of whether development within this group can at all be related to the main patterns of development seen in other conidial fungi.

2. Material and methods

The descriptions below are based upon examination of material in the herbarium of the Commonwealth Mycological Institute (IMI). All specimens were examined by light microscopy, critical observations being made using differential interference contrast optics. Many slide preparations already in existence and mounted in a variety of media were examined to assess what information was available to earlier workers on this group. New slides were also prepared, mounted in erythrosin in ammonia (Sutton 1980). In the following list of specimens examined, the annotation "(T)" indicates that a particular specimen is type material (*e.g.* holotype, isotype, lectotype etc.) for the specific epithet under which it is listed. Specimens listed under a teleomorphic name relate solely to the teleomorph. Specimens listed under an anamorphic name may relate to more than one anamorph. The same specimen may therefore be listed in two places, *i.e.* as a teleomorph and as an anamorph.

2.1 *Teleomorphs*

Apiospora camptospora Penzig & Saccardo, IMI 79872; *A. montagnei* Saccardo, IMI 13421, 39081, 79869, 262041; *Ceratocystis adiposa* (Butler) C. Moreau, IMI 21355, 159574; *C. fimbriata* Ellis & Halstead, IMI 47410; *Chaetosphaeria cupulifera* (Berkeley & Broome) Saccardo, IMI 29343c, 70216(T); *Khuskia oryzae* H. Hudson, IMI 79239(T).

2.2 *Anamorphs*

Arthrinium aureum Calvo & Guarro IMI 238036(T), 252326d; *A. caricicola* Kunze, IMI 19517, 19518, 19520, 19521, 19522, 19523, 19527, 69721(T); *A. curvatum* Kunze var. *minus* M. B. Ellis, IMI 19424, 19582, 19589, 21201a(T), 23211a, 27814, 237621; *A. cuspidatum* (Cooke & Harkness) Tranzschel, IMI 6964(T), 6966, 7076, 19442, 19449, 19450, 19565, 113760; *A. euphorbiae* M. B. Ellis, IMI 110788(T), 151825, 216361, 285638b; *A. fuckelii* Gjaerum, IMI 96384; *A. kamtschaticum* Tranzschel & Woronich., IMI 117078(T), 125200, *A. lobatum* M. B. Ellis, IMI 74782(T); *A. luzulae* M. B. Ellis, IMI 96386(T), 99595, 101236, 129233; *A. morthieri* Fuckel, IMI 19560(T), 170176, 216360; *A. muelleri* M. B. Ellis, IMI 173321(T); *A. phaeospermum* (Corda) M. B. Ellis, IMI 56361, 63333, 112921, 113076, 155848, 171440, 184136, 202657, 248559 and many more; *A. puccinioides* (DC) Kunze, IMI 395, 1584b, 17326, 17326a, 19575, 19628, 19631, 123397 and many more; *A. sacchari* (Spegazzini) M. B. Ellis, IMI 37924e, 93882a, 98841, 101676(T), 199234 and many more; *A. saccharicola* Stevenson, IMI 74759c; *A. spegazzinii* Subramanian, IMI 101677(T), 216805; *A. sphaerospermum* Fuckel, IMI 103478(T); *A. sporophleum* Kunze, IMI 20967, 27815, 264419; *A.* state of *Apiospora montagnei*, IMI 79869, 182296, 184251, 187822, 194057, 194452, 203260, 205996, 231470 and many more; *A. urticae* M B Ellis, IMI 8936(T); *A. ushuvaiense* Spegazzini, IMI 75583, 101668(T).

Ascoconidium tsugae Funk, IMI 118039(T); *Bloxamia bohemica* Minter & Holubová-Jechová, IMI 243630, 243651(T), 243745, 243752, 243883, 247620; *B. leucophthalma* (Leveille) Höhnel, IMI 227912, 277790; *B. sanctae-insulae* Minter & Coppins, IMI 245014; *Catenularia* state of *Chaetosphaeria cupulifera*, IMI 18044(T), 29343c, 70216(T); *Chaetochalara africana* B. Sutton & Pirozynski, IMI 94904(T), 263238; *C. bulbosa* B. Sutton & Pirozynski, IMI 89645a(T); *C. cladii* B. Sutton & Pirozynski, IMI

10171(T), 89626; *Chalara thielavioides* (Peyronel) Nag Raj & Kendrick, IMI 79278, 153626, 280528; *C.* state of *Ceratocystis adiposa*, IMI 21285, 21355, 159574, 192094, 207358, 254176, 281346, 286069; *C.* state of *Ceratocystis fimbriata*, IMI 8764, 47410, 209781, 217432; *C.* state of *Ceratocystis paradoxa* (Dade) C. Moreau, IMI 15302, 198806, 223309, 229489, 234000; *Conioscypha hoehnelii* P. M. Kirk, IMI 249629(T); *C. lignicola* Höhnel, IMI 270439, 270440; *C. varia* Shearer, IMI 147409; *Conoplea elegantula* (Cooke) M B Ellis, IMI 107638; *C. fusca* Persoon, IMI 69978, 209220f, 212865; *C. globosa* (Schweinitz) Hughes, IMI 33163; *C. juniperi* Hughes, IMI 33157; *C. mangenotii* O Reisinger, IMI 179344, 276351, 276370; *C. olivacea* Fries, IMI 33133; *Cordella clarkii* M B Ellis, IMI 171096(T); *C. coniosporioides* Spegazzini, IMI 69877(T), 74789.

Dictyoarthrinium africanum Hughes, IMI 38596d(T); *D. rabaulense* Matsushima, IMI 51264, 104096, 119899, 148935, 181940; *D. sacchari* (Stevenson) Damon, IMI 37924b, 170212, 177858, 214813, 222849, 224333, 225218; *Echinobotryum atrum* Corda, IMI 1370, 14030, 61840, 76568a, 76568b; *Endocalyx cinctus* Petch, IMI 287889; *E. indumentum* Okada & Tubaki, IMI 287888; *E. melanoxanthus* (Berkeley & Broome) Petch var. *grossus* Okada & Tubaki, IMI 287881, 287883; *E. melanoxanthus* var. *melanoxanthus*, IMI 287873, 287875, 287877, 287878, 287879; *E. thwaitesii* Berkeley & Broome, IMI 48588(T), 263414; *Gamsia dimera* (W. Gams) Morelet, IMI 111371; *Gilmaniella humicola* Barron, IMI 92086, 101206(T), 102286, 180572, 192376, 193035; *Humicola fuscoatra* Traaen, IMI 4292(T), 250484; *H. grisea* Traaen, IMI 213014; *Mammaria echinobotryoides* Cesati, IMI 69710(T), 76107a, 76598, 83441, 90616, 92739, 100815, 107675, 153657; *Mammaria* sp., IMI 269030; *Megalododochium elaidis* (Beeli) Deighton, IMI 46626, 48970a, 56737a, 113675(T); *Melanographium citri* (Fragoso & Ciferri) M B Ellis, IMI 39940c, 46707b, 59270; *M. cookei* M B Ellis, IMI 4300(T), 7348, 46528; *M. fasciculatum* Hughes, IMI 99979, 174790; *M. selenoides* (Saccardo & Paoletti) M. B. Ellis, IMI 69379, 75674(T), 99978; *M. spinulosum* (Spegazzini) Hughes, IMI 69878, 69879(T); *M. trachycarpi* Hino & Katumoto, IMI 99960(T); *Nigrospora gerlenkoanum* Novobremova, IMI 174726(T); *N. panici* Zimmermann, IMI 60675, 78285b; *N. oryzae* (Berkeley & Broome) Petch, IMI 171270, 171281; *N. vietnamensis* Holubová, IMI 99670(T); *N.* state of *Khuskia oryzae*, IMI 79239(T), 192978, 195440, 208372, 209580, 211940; *N.* sp., IMI 256852.

Paathramaya sundara Subramanian, IMI 62748(T), 152552, 160059, 162553, 170661 and more; *Phialocephala bactrospora* Kendrick, IMI 47572, 52633; *P. dimorphospora* Kendrick, IMI 144474, 194266; *P. fusca* Kendrick, IMI 56093b; *P. humicola* Jong & Davis, IMI 173641(T); *Pteroconium intermedium* M B Ellis, IMI 32025(T), 231478, 231482; *P. pterospermum* (Cooke & Massee) Grove, IMI 57982; *P.* state of *Apiospora camptospora*, IMI 79871, 79872, 79873, 203097, 234468, 240297b, 285834; *P.* sp., IMI 234989; *Spegazzinia deightonii* (Hughes) Subramanian, IMI 3792a, 38493b, 38568b(T), 48047; *S. intermedia* M B Ellis, IMI 119898(T), 139433; *S. lobulata* Thrower, IMI 49132(T); *S. parkeri* Sivasithamparam, IMI 173948(T); *S. sundara* Subramanian, IMI 183791, 185949, 186911, 187797, 210143; *S. tessarthra* (Berkeley & Curtis) Saccardo, IMI 167078, 216589, 224161; *Sporoschisma juvenile* Boudier, IMI 27356b, 27358, 163738; *S. mirabile* Berkeley & Broome, IMI; *Thielaviopsis basicola* (Berkeley & Broome) Ferraris, IMI 742(T), 35618, 179688, 262289b, 278656; *Wardomyces anomalus* Brooks & Hansford, IMI 25846(T), 32064, 76597, 100637; *W. humicola* Hennebert & Barron, IMI 98886(T), 144473; *W. inflatus* (Marchal) Hennebert, IMI 98609, 98885, 105577, 234312; *W. ovalis* W. Gams, IMI 117372(T), 217208, 223228; *W. pulvinatus* (Marchal) Dickinson, IMI 96467, 98294, 231921.

3. Analysis of *Arthrinium*

3.1 *Conidial development*

On their natural substrata, species of *Arthrinium* develop conidia in the following manner: the first conidium is initiated holoblastically, presumably by a wall building apex (Minter *et al* 1983a), from a small usually swollen conidiogenous cell attached to the vegetative hyphae (figure 1a). The apical wall building appears to be accompanied by diffuse wall building since the conidium swells as it grows. It matures rapidly, becoming pale to dark brown, and is delimited by what appears to be a two-ply septum (figures 1b, c). It then partially secedes by fracture of the outer cell wall at the level of the delimiting septum (figure 1d), the fracture apparently being caused by pressure from an enteroblastic proliferation which is itself brought about by the activity of a replacement wall building apex. The conidium is then carried away from its conidiogenous cell by the proliferation growth which takes the form of an apparently highly specialised hyaline hypha (figures 1d, e). Because the proliferation is enteroblastic, a small discontinuity can usually be observed with the light microscope between the top of the conidiogenous cell and the bottom of the growing hypha (figure 1e, arrow).

The hypha grows continuously from its base and is never branched. It seems likely, therefore, that the replacement wall building apex by which it was initiated is rapidly converted into a wall building ring (Minter *et al* 1983a) located just below the top of the conidiogenous cell (figure 1f, arrow). As a result, the highest point of the hypha, *i.e.* where it is attached to the first conidium, is its oldest part. As the hypha grows, septa are laid down (figures 1f, g), so that eventually the first conidium may be separated from its conidiogenous cell by a considerable number of cells (figure 1g). Before this point is reached, however, it is normal for most of the cells in the specialised hypha to produce more conidia of their own, in a roughly ordered succession, according to the age of the cells, the oldest first (figure 1h–k). Each cell usually produces between one and five conidia apparently by a series of replacement wall building apices (Minter *et al* 1983a). In classical descriptions of these fungi, the specialised hypha is called a basauxic conidiophore, and the first conidiogenous cell (figure 1k, arrow) is a conidiophore mother cell.

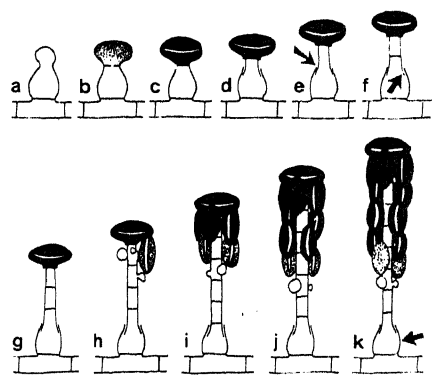


Figure 1. Development of *Arthrinium* species (semi-diagrammatic).

3.2 *Features of the conidia*

On their natural substrata conidia of all species of *Arthrinium* are moderately to very deeply pigmented and aseptate. In many species the conidia are lenticular in shape (figure 2a), but in some the shape varies from this norm, and conidia are also known which are, for example, elongated ellipsoidal (figure 2b), polygonal (figure 2c), cashew-nut shaped (figure 2d), rectangular in face view (figure 2e) or even reminiscent of the shape of Napoleon's hat inverted (figure 2f). In most species conidia are smooth when viewed with the light microscope (figures 2a–f), but in a few they are minutely ornamented to distinctly roughened (figures 2g, h).

Germ slits are very frequent except on conidia with rough walls where they may simply be hard to detect with the light microscope. In most species, treating the point of attachment of the conidium as a pole, the germ slit is oriented more or less equatorially. In many species pores can also be observed. In some species up to four pores may be present on each conidium in addition to a germ slit (figure 2a). In others only one pore is present, and is best observed in the seceded conidium at the point where it had been attached (figures 2b, f). With careful observation, a small darkened circle can sometimes be seen around this pore in seceded conidia (figure 2f) apparently marking the fractured outer cell wall of the two-ply septum broken during secession. A corresponding circle is also frequently observed after secession on the conidiogenous cell (figure 5c, arrowhead), suggesting that secession is schizolytic. Since conidia of *Arthrinium* are violently discharged (Webster 1966), a rather infrequent feature among hyphomycetes, it is possible that the secessional mechanism is not straightforward. The mechanisms by which conidia of these species disperse are also poorly known, though it has been reported that insects, slugs, snails and molluscs may at times be involved (Ellis *et al* 1951).

Many species of *Arthrinium* also produce apparently deformed conidia which remain a paler brown and which often contain a large refractive body (figure 3). The first conidium to be produced on a given conidiophore is often like this. Such conidia tend to be produced more commonly towards the end of a growing season and it has been surmised that these have a function of storage of metabolic waste products (Ellis

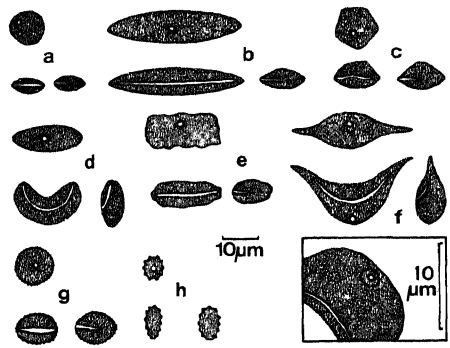


Figure 2. Conidia of various species of *Arthrinium*: **a.** A state of *Apiospora montagnei*; **b.** *A. caricicola*; **c.** *A. puccinioides*; **d.** *A. kamtschaticum*; **e.** *A. fuckelii*; **f.** *A. cuspidatum* (inset shows detail of conidial pore and secessional scar); **g.** *A. aureum* (the minute roughening on these conidia is exaggerated in this illustration); **h.** *A. spegazzinii*

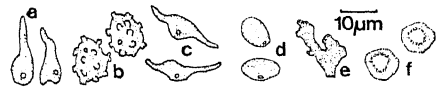


Figure 3. Apparently deformed conidia of various species of *Arthrinium*: **a.** *A. fuckelii*; **b.** *A. lobatum*; **c.** *A. caricicola*; **d.** *A. cuspidatum*; **e.** *A. sporophleum* (in pure culture); **f.** *A. sporophleum* (*in vivo*).

et al 1951). These anomalous conidia may take a remarkable range of shapes (figures 3a–f) though the shape is usually constant and often characteristic for a species. Like the properly formed conidia, these deformed conidia may have pores, though not in general germ slits. The proportion of normal to deformed conidia also varies between species.

3.3 *Features of the first conidiogenous cell (conidiophore mother cell)*

On their natural substrata in most species of *Arthrinium* this cell is small, inconspicuous and lightly pigmented. It is usually more or less globose, but in a few species, notably *A. phaeospermum*, it may be lageniform with a more or less well-defined venter and neck (figure 4a). Periclinal thickening may often be observed below the opening of the cell (figures 4b, c), but transmission electron micrographs so far published show no evidence of an accumulation of cell walls sequentially laid down (Cole and Samson 1979) such as are encountered in conidiogenous cells of fungi such as *Trichoderma saturnisporum* Hammill. The absence of such an accumulation, together with the appearance of the specialised hyphae arising from such cells, is interpreted here as evidence that the specialised hypha in this group is produced by the wall building ring mode of development (Minter *et al* 1983a).

In some collections the proliferation giving rise to the specialised hypha appears not to be enteroblastic since (at least at the level of the light microscope) no discontinuity can be observed at the top of the first conidiogenous cells (conidiophore mother cell) (figure 4d). In other collections it appears that a substantial part of the original outer wall of the first conidiogenous cell is taken away with the first conidium when enteroblastic proliferation occurs to form the specialised hypha, so that the original pigmented outer wall of the first conidiogenous cell ends abruptly less than halfway up that cell (figure 4e).

3.4 *Features of the specialised hypha (basauxic conidiophore)*

Species of *Arthrinium* can be divided into two groups according to the type of specialised hypha (basauxic conidiophore) produced. In one group the specialised hypha is comparatively broad, with many septa close together, and these septa are greatly thickened, blackened and rather refractive, making them a most prominent feature (figures 5a–c) which can often be observed at an early stage of development, even within the first conidiogenous cell (conidiophore mother cell) as they are being laid down (figure 5a, arrow). In specialised hyphae of this type, the individual hyphal cells are broadly cylindrical and straight and may have walls which are thinnest near the septum at each end, and which thicken markedly towards the mid-point of the cell, so that in side view the protoplasm can appear to occupy two conical regions in the cell, one upper and the other lower (figure 5a, arrowheads), although often the cell wall is

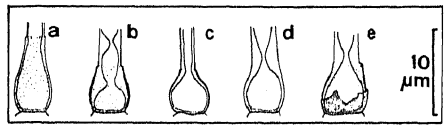


Figure 4. Examples of conidiophore mother cells: **a.** *A. phaeospermum*; **b.** *A. cuspidatum*; **c.** *A. aureum*; **d.** *A. caricicola*; **e.** *A. caricicola.*

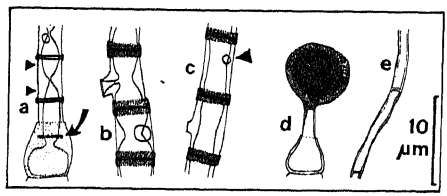


Figure 5. Examples of specialised hyphae: **a.** *A. sporophleum*; **b.** *A. cuspidatum*; **c.** *A. caricicola*; **d.** *A. aureum*; **e.** *A. aureum.*

uniformly thick. Because specialised hyphae of this sort are comparatively broad, the act of producing a succession of conidia does not cause them to take on a zig-zagged appearance.

In the other group the specialised hypha is narrower and the septa are few and far between and not greatly thickened or blackened (figures 5d, e). The individual cells of such hyphae are narrowly cylindrical and flexuous and show no perceptible thickening of their walls. The narrow nature of these specialised hyphae means that they are often rather zig-zagged in appearance after producing several conidia, as though they were sympodial conidiophores in reverse. Members of this second group have sometimes been put in a separate genus, *Papularia* Fries. Many mycologists have found it difficult to decide whether this distinction in types of specialised hyphae is a valid reason for separating these groups into separate genera, largely because the significance of the phenomenon has apparently never been explained.

3.5 *Appearance in pure culture*

Most species which have been isolated into pure culture produce a lot of their conidia on agar in a manner indistinguishable from that of the natural substratum. It is by no means uncommon, however, also to encounter conidia produced by a variety of other developmental sequences. Many of these apparently abnormal sequences are shared by a lot of the species known in pure culture, although the proportion of conidia so produced varies considerably from one isolate to another, or indeed within one isolate subcultured onto different media.

Conidia may be produced holoblastically from a first conidiogenous cell but with no subsequent proliferation to produce a specialised hypha (figure 6a), or they may arise holoblastically direct from an apparently unmodified vegetative hypha (figure 6b). In at least one species, conidia have been observed produced on conidiogenous cells arising directly from ascospores placed on agar (Hudson 1963a). The conidia may have shapes different from that on the natural substrate (figures 6c–e) and sometimes

Figure 6. Examples of conidia of *Arthrinium* species produced in culture (all drawn from *A.* state of *Apiospora montagnei*).

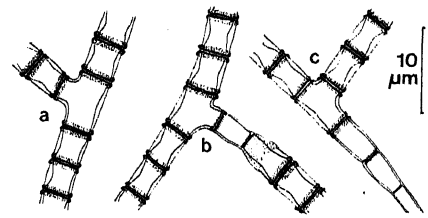


Figure 7. Examples of variation in broad specialised hyphae of *Arthrinium* species produced in culture: **a.** branched hypha with dark septa; **b.** branched hypha with dark septa giving rise to intercalary conidiophore mother cell; **c.** dark septa intergrading with ordinary septa.

even may be produced by the laying down of pigment and sometimes a germ slit in a terminal or intercalary cell of an apparently unmodified vegetative hypha (figures 6f–h).

Species which produce broad specialised hyphae (basauxic conidiophores) with dark septa on their natural substrates may often fail to produce such hyphae in pure culture. Instead, their conidia are borne holoblastically from hyphal systems which are branched and have no conidiophore mother cells. The presence of branching suggests that such systems have probably been produced by apical and not ring wall building. It is very common, however, for these hyphal systems to bear the darkened septa so characteristic of the specialised hyphae (figures 7a–c), and occasionally they may also bear conidiophore mother cells giving rise to specialised hyphae growing by ring wall building exactly the same as *in vivo* (figure 7b). When the hyphal systems bearing typical darkened septa are traced back, it is often found that there is an intergradation between the darkened septal type and septa typical of apparently normal vegetative hyphae (figure 7c). Conidia delimited by darkened septa are also occasionally seen.

4. Other genera of the *Arthrinium* group

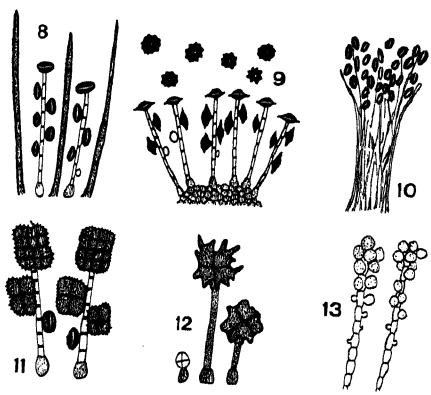
Other genera in this group are on the whole rather similar to *Arthrinium*. *Cordella* Spegazzini (figure 8) differs because it produces setae among the clusters of specialised hyphae. *Pteroconium* Saccardo ex Grove (figure 9) is sporodochial and has rather spiky conidia in at least one species, *P. pterospermum*. The one species known in pure culture, *P.* state of *Apiospora camptospora*, shows a similar range of forms to that described above for isolates of *Arthrinium*. *Endocalyx* Berkeley & Broome (figure 10) produces its specialised hyphae in a complex structure reminiscent of a discomycete fruitbody and, while still producing unbranched specialised hyphae, apparently never produces the first conidiogenous cell (conidiophore mother cell) (Okada and Tubaki 1984). Okada and Tubaki (1984) also demonstrated that conidia of this genus may vary by species

from almost smooth to highly ornamented. In species with highly ornamented conidia they did not observe germ slits or germ pores. In their isolates of these fungi a similar range of forms to those found in isolates of *Arthrinium* may be found, and in particular they noted the presence of conidia produced by the laying down of pigment in terminal or intercalary cells of apparently unmodified vegetative hyphae.

Dictyoarthrinium Hughes (figure 11) produces conidia which are multiseptate and rather roughened. In their early stages of development conidia of this genus are generally lenticular, aseptate, brown, and have a germ slit and one or more pores. In pure culture some species of this genus show the production of branched hyphal systems with darkened septa very well. *Spegazzinia* Saccardo (figure 12) never produces more than one conidium from each first conidiogenous cell, so that the specialised hypha appears simply to serve the purpose of lifting this unique spore a little higher than would otherwise have been the case. Conidia of *Spegazzinia* species, like those of *Dictyoarthrinium*, are multiseptate and roughened, but they differ because they are usually dimorphic and can still be hyaline at their four-cell stage. In species where the conidia are dimorphic, one type of conidium is smooth or less spiny than the other and is borne on a shorter specialised hypha. The specialised hyphae in this genus also appear rather different from those of the other genera discussed above, since they are pigmented and usually lack septa. Species of *Graphiola* Poiteau (figure 13) have been thought by some to belong in this group (*e.g.* Hughes 1953), but Cole (1983) has demonstrated that they are truly basidiomycetes.

5. Known synanamorphs and teleomorphs of the *Arthrinium* group

Apart from the report by Okada and Tubaki (1984) of darkened intercalary conidia in cultures of *Endocalyx* species discussed above, there are apparently no published reports of synanamorphs for species of this group. Among the specimens of *A. puccinioides* and *A. sporophleum* in Herb. IMI were cultures purporting to have originated from conidia of these species and producing conidiomata of a species of *Hendersonia* Saccardo or *Phaeoseptoria* Spegazzini. At least one of the slide preparations of these conidiomata was, however, labelled "contaminant", though this



Figures 8–13. Other genera of the *Arthrinium* group (semi-diagrammatic): **8.** *Cordella*; **9.** *Pteroconium*; **10.** *Endocalyx*; **11.** *Dictyoarthrinium*; **12.** *Spegazzinia*; **13.** *Graphiola*.

label itself had been crossed out again. It seems therefore only sensible to reject this unpublished record of a possible synanamorph.

The few teleomorphs known for this group all belong in *Apiospora* Saccardo (Hudson 1963a) and *Physalospora* Niessl (Arx 1974). *Pseudoguignardia* Gutner has been cited as a teleomorph (Cooke 1954), but Müller and Arx (1962) regarded this genus as synonymous with *Physalospora*, and the teleomorph connexion in *Physalospora* cited above probably refers to the same record. The illustration of this *Physalospora*/*Pseudoguignardia* teleomorph by Ellis *et al* (1951) shows a species with aseptate ascospores becoming 1-septate, with the septum located in the lower part of the ascospore. This is an unusual feature which is typically found in species of *Apiospora*, suggesting that the teleomorphs of this group are probably closely related.

The three genera, *Apiospora, Physalospora* and *Pseudoguignardia* are all placed in the Amphisphaeriaceae, which is treated as one of the four families of the Sphaeriales (= Xylariales) in the seventh edition of Ainsworth and Bisby's Dictionary of the Fungi (Hawksworth *et al* 1983). The Amphisphaeriaceae at present scarcely represent a phylogenetically uniform and well-understood family: most of the anamorph-teleomorph connexions listed for this family by Kendrick and DiCosmo (1979) are so different from the *Arthrinium* group that any meaningful relation between them seems most unlikely. The list does, however, provide one useful lead: in at least one of the genera, *Apiospora* (under its synonym *Khuskia* H. Hudson), a different anamorph is known (Hudson 1963b), and this anamorph is a species of *Nigrospora* Zimmermann.

6. Comparison of *Nigrospora* and *Arthrinium*

Nigrospora is at present commonly regarded as being completely different in development from *Arthrinium* and its related genera. Ellis (1971), for example, in his monumental work, followed the conventional classification of Kananaskis (Kendrick 1971), and distinguished *Nigrospora* from *Arthrinium* because it has conidiogenous cells which each normally produce only one conidium, and do not proliferate to produce a "basauxic conidiophore". As a result, Ellis separated *Nigrospora* from *Arthrinium* by literally hundreds of pages and scores of genera! The fact that he also separated them in his key at the eleventh dichotomy out of more than two hundred is a strong indication of how much these fungi have been believed to differ.

When the *Nigrospora* state of *K. oryzae* (figure 14) and other species of the genus are compared with species of *Arthrinium*, however, there are many clear and evident similarities. In both genera the species produce deeply pigmented conidia, each with a germ slit and a small pore marking the point of attachment to the conidiogenous cell. Both genera are among a very small number of hyphomycetes with the unusual feature of having a violent conidial discharge mechanism (Webster 1952) (n.b. this does take a slightly different form in some species of *Nigrospora* which have a special vesicle surrounding the septum delimiting the conidium from its conidiogenous cell (figure 14, arrow)). In pure culture some species of *Nigrospora* sometimes show the same sorts of apparently abnormal conidia as are found in *Arthrinium* species.

The difference in development between the two genera, *i.e.* the fact that *Arthrinium* species have "basauxic conidiophores" while those of *Nigrospora* do not, only becomes apparent after the first conidium has been fully formed. As a result, many experienced mycologists experience practical difficulties in telling them apart! All this, coupled with

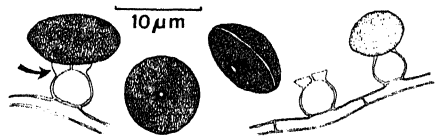


Figure 14. *Nigrospora* state of *Khuskia oryzae.*

the fact that both anamorphic genera share the same teleomorphic genus, suggests strongly that *Arthrinium* and its related genera may not be as different from other hyphomycetes as the conventional classifications of Hughes (1953), Kananaskis (Kendrick 1971) and Ellis (1971) might indicate.

7. Re-assessment of the developmental characteristics exhibited by *Nigrospora*, *Arthrinium* and related genera

All conidia of fungi discussed in the foregoing paragraphs are apparently produced by the activity of a modified hyphal tip (wall building apex). Given our current state of knowledge (Gooday 1984), this activity probably involves ultrastructural vesicles clustered at the tip and laying down new wall material. There is no experimental evidence for these vesicles in the genera under discussion, but their presence has been demonstrated in a wide range of fungi wherever hyphal tip growth has been investigated. Indeed it seems not unlikely that the wall building apex mode of growth is virtually universal in higher fungi.

The wall building ring mode of growth is also apparently present in many of these fungi. Like the wall building apex, the wall building ring involves vesicles, although these vesicles are differently distributed in the cell (Minter *et al* 1983a). The wall building ring has also not been demonstrated experimentally in this group, although the circumstantial evidence for it seems good. On its own, however, it is certainly not a characteristic on which to place great emphasis. It is absent from species of *Nigrospora*, which have teleomorphs closely related to those of *Arthrinium* species, and is present in a wide range of other fungi with teleomorphs far removed from those of *Arthrinium*. Indeed, it is probably present in species of *Graphiola*, a genus now regarded as belonging in the basidiomycetes (Cole 1983).

During their development, all of these fungi exhibit holoblastic growth. This form of hyphal growth is completely widespread: it is hard to conceive of a higher fungus not exhibiting holoblastic growth at most points of its development. All the term means is that between any two stages of development there is continuity of all the cell wall layers involved (Minter *et al* 1982). In most of these fungi, an enteroblastic phase can also be detected. This is equally unsurprising. Enteroblastic development is when there is no continuity of the outermost wall layer or layers between any two stages of development (Minter *et al* 1982). It occurs in an extremely wide range of fungi, for example, when fresh hyphal growth breaks out of an old hypha with hardened walls. In some fungi the enteroblastic mode appears to be under genetic control, *e.g.* enteroblastic proliferation in many 'phialides' and 'annellides'. Much of the enteroblastic development observed in the group under discussion fits into this category, which nevertheless embraces a depressingly wide range of fungi.

At first sight the dark septa seem attractive characteristics, but as they intergrade in

culture with normal septa their use as criteria is limited. Furthermore, absolutely nothing is known about how these special dark septa are produced, and without ultrastructural evidence there seems to be no justification for claiming that such septa are fundamentally different from normal deuteromycete septa. The septa delimiting conidia in these fungi, and enabling those conidia to secede, also seem to be no different from those of innumerable other deuteromycetes which have schizolytic secession.

Almost all of our interpretation of conidial development, therefore, relates to how walls of conidia and their conidiogenous cells are formed. Events occurring in the protoplasm are still generally unavailable as taxonomic criteria. Knowledge of how cell walls are laid down is still mainly the preserve of the developmental mycologist and, even if available, would probably be impossible to put to practical use in taxonomy. It is clear, however, that conidia and conidiogenous cells are little more than modified hyphae. It is also clear that there is a very limited number of ways in which hyphae can be produced. The very building blocks on which the conventional taxonomy of these fungi is based, *i.e.* holoblastic and enteroblastic development etc., are therefore so general among the higher fungi that one could even assert that any fungus producing branched and septate hyphae has the genetic potential to produce conidia by a replacement wall building apex system.

It seems to follow that the holoblastic sympodial mode of development, for example, has probably evolved many times. The enteroblastic percurrent mode found in the so-called annellides is scarcely more complex. The replacement wall building apex 'phialide' is no more than a rather well-synchronised annellide. The wall building ring mode occurs in 'phialides', 'meristem arthrospores', 'conidiophore mother cells' and elsewhere in very diverse fungi. The implication is that all of these forms too are polyphyletic.

It is therefore scarcely surprising that the conventional systems of classifying hyphomycetes on the basis of development have encountered problems. They have laid too much stress on single characteristics which are each almost universal among higher fungi, and they have used clusters of these characteristics as the bases for major groups (thallic, blastic, annellidic, phialidic etc.) without sufficient critical analysis of each characteristic individually. The problem here is how to relate *Arthrinium* to other deuteromycetes in a way which avoids these pitfalls! No individual characteristic must be stressed, and each must be assessed on its own critically, and related in some rational manner to the other characteristics. An attempt will now be made to do this by means of a hypothesis explaining the development of *Arthrinium*.

8. Hypothesis

Suppose that species of *Arthrinium* have evolved from an ancestor which produced two types of conidia, one hyaline and the other dark, but which did not have the so-called conidiophore mother cells or basauxic conidiophores. A hypothetical life-cycle of this ancestor, ignoring any teleomorph for simplicity, is shown in figure 15. It will become apparent later in this paper that a number of fungi exists with life-cycles exactly like this hypothetical ancestor. Hyaline conidia (figure 15a) disperse, find substrate, germinate (figure 15b) and produce a mycelium (figure 15c). Conidiogenous cells on this mycelium produce dark conidia (figure 15d), which disperse (figure 15e), find a substratum, germinate (figure 15f), produce a mycelium (figure 15g), which in turn produces conidiogenous cells of the hyaline conidial state (figure 15h). Of course dark

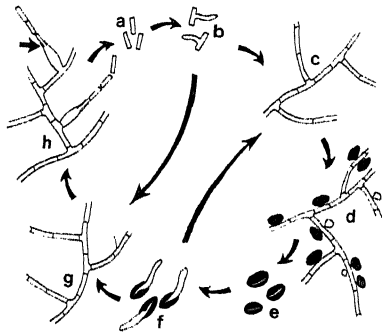


Figure 15. Life-cycle of a hypothetical ancestor of *Arthrinium*.

conidia may produce a mycelium giving rise to further conidiogenous cells of the dark conidial state, and hyaline conidia may produce a mycelium giving rise to further conidiogenous cells of the hyaline conidial state (figure 15, short cut arrows).

Since species of *Arthrinium* show the wall building ring mode of development at one stage of their development, the hypothetical ancestor has also been shown with a wall building ring stage (figure 15h, arrow). In the ancestor, this stage has been placed in the conidiogenous cells of the hyaline conidial stage since, according to the hypothesis, there is no conidiophore mother cell in which to place it. The hypothetical ancestor is therefore like many real hyphomycetes which have wall building rings giving rise to hyaline conidia. The dark conidia of the hypothetical ancestor have been shown as smooth and with a germ slit, but from our analysis of *Arthrinium* and its relatives, roughened dark conidia, with or without germ slits and/or pores might equally well be expected.

If the hypothesis is correct, then species of *Arthrinium* must have life-cycles which are in some way reduced. To achieve this a degree of developmental plasticity might well be expected in these fungi. This could well explain the plasticity observed in their cultures and commented on above. The question of which parts of the ancestral life-cycle were lost must now be addressed. Consider the possibility that the ancestor lost the ability to produce, disperse and germinate hyaline conidia (ancestral stages; figures 15a, b). This is very plausible in view of the fact that hyaline conidial synanamorphs are not known for *Arthrinium* species. In a hypothetical life-cycle so reduced (figure 16), the conidiogenous cells of the erstwhile hyaline conidial stage go directly to producing the mycelial stage analogous to that of figure 15c. The similarity of development in figure 16 to that of *Arthrinium* species of the *Papularia* group (figures 5d, e) is startling.

Consider instead the possibility that the ancestor lost the ability to produce mycelium and dark conidial stage conidiogenous cells (ancestral stages: figures 15c, d) following germination of the hyaline conidia. This would result in the hyaline conidia germinating to produce dark conidia immediately (figure 17). At first sight this mode of development bears little resemblance to that of *Arthrinium* species. The production of two conidial stages adjacent in the life-cycle might, however, seem unlikely. One of them would probably lose its dispersal function. This would mean that the conidia of one of the stages involved would not secede, and one would expect to see an alteration in the secession mechanism associated with the loss of a dispersal function. If the dispersal function of hyaline conidia of the ancestor were lost, one would expect them to adhere in chains, bearing the dark conidia on them (figure 18). The similarity of

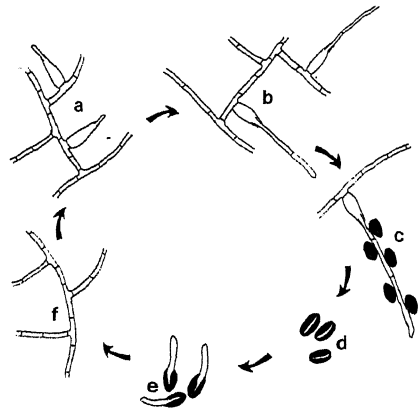


Figure 16. A possible reduced life-cycle of the hypothetical ancestor of *Arthrinium*.

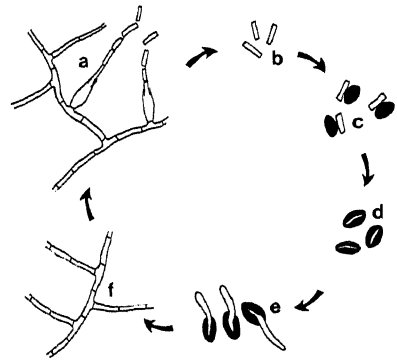


Figure 17. A second possible reduced life-cycle of the hypothetical ancestor of *Arthrinium*.

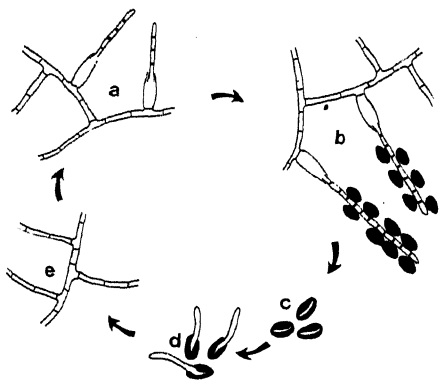


Figure 18. A third possible reduced life-cycle of the hypothetical ancestor of *Arthrinium*.

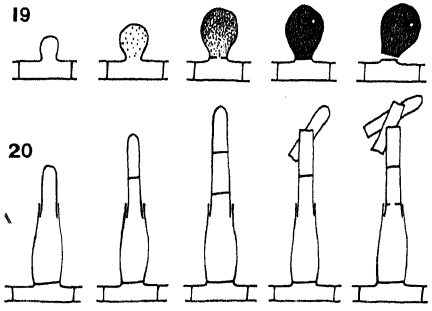
development in figure 18 to that of *Arthrinium* species with the broad specialised hyphal type (figures 5a–c) is also startling.

9. Testing the hypothesis

The test of any scientific hypothesis lies in its ability to predict (Popper 1959). It is therefore interesting to offer the following predictions based on this hypothesis: (1) fungi will be found which have life cycles similar to the hypothetical ancestor of *Arthrinium* species; (2) such fungi will have both hyaline and dark conidial stages; (3) many of the dark conidial stages will involve germ slits and/or pores; (4) if these are absent, the dark conidia will tend to be rough walled; (5) variation between smooth and rough dark conidial stages will be found within clusters of related fungi (and this will be a characteristic of approximately specific significance, just as in *Arthrinium* species); (6) related fungi will show a similar plasticity of development (varying freely between holoblastic and enteroblastic growth modes, for example) and many will also have reduced life-cycles (but different parts of the ancestral life-cycle will have been lost); (7) dark septa will occur in at least some of the related fungi, particularly where there is no obvious hyaline conidial stage, often on branched hyphal systems (no more than a reflexion of the sort of developmental plasticity found in species of *Arthrinium* in pure culture); (8) intermediates will be found between species with dispersing hyaline conidia and species with these conidia tightly bound by dark septa (*e.g.* species with hyaline conidia which are delimited by dark septa and nevertheless secede); (9) at least some evidence will be found for an intermediate stage where hyaline conidia disperse and immediately give rise to dark conidia. Some fungi providing examples of all of these predictions will now be considered.

9.1 *Chalara thielavioides*

The development of the dark conidium stage of *C. thielavioides* is shown semi-diagrammatically in figure 19. The conidiogenous cell is hyaline and is usually little differentiated from the vegetative hyphae. The conidium is initiated holoblastically by apical wall building, with some synchronous diffuse wall building, and turns dark brown. As it changes colour it is delimited by a presumably two-ply septum, and it becomes rather thicker-walled than the conidiogenous cell which bore it. With careful



Figures 19, 20. Development of the **19.** dark and **20.** hyaline conidial stages of *C. thielavioides*.

observation a small pore can be seen in many mature conidia, although this pore is not located at the delimiting septum. Secession is schizolytic, and the conidiogenous cell does not normally proliferate to produce more than one conidium.

The development of the hyaline conidium stage of the same species is shown semi-diagrammatically in figure 20. The conidiogenous cell is hyaline and may be slightly swollen compared to undifferentiated vegetative hyphae. A succession of conidia is produced enteroblastically by means of a wall building ring system. The conidia are arranged in unbranched true (Subramanian 1972) chains which hold together rather weakly, and are cylindrical, being delimited by two-ply septa and seceding schizolytically.

Taken together, figures 19 and 20 show that *C. thielavioides* has a life cycle very similar to that of the hypothetical ancestor of *Arthrinium* species (prediction 1 of the hypothesis). The fungus has two different conidial stages, one dark and the other hyaline (prediction 2). The hyaline conidia are produced by a wall building ring system, while the dark conidia have germ pores (prediction 3). The rather well-developed collarette of the hyaline stage conidiogenous cells appears at first sight different from the apex of the so-called conidiophore mother cells in *Arthrinium*, but one might expect such a difference in view of the fact that the conidiogenous cell in *C. thielavioides* is producing spores for dispersal (which must be 'encouraged' to form chains by the long collarette), whereas the conidiophore mother cells of *Arthrinium* species are producing a non-caducous chain of cells which have therefore no need of a collarette to keep them in line.

Most remarkable of all, however, is the fact that, in pure culture, *C. thielavioides* exhibits a plasticity of development similar to that encountered in species of *Arthrinium*. In the example shown in figure 21, one of the hyaline conidia in a chain has germinated to produce, immediately, a dark conidium even before it has itself seceded from its chain. This is an excellent example of the potential for reduced life cycles as a result of developmental plasticity (prediction 6). The developmental similarity between *C. thielavioides* as seen in figure 21 and species of *Arthrinium* is striking, and is strongly suggestive that *C. thielavioides* represents an intermediate stage where hyaline conidia immediately give rise to dark conidia (prediction 9).

9.2 *Certain other species of Chalara (Corda) Rabenhorst*

The *Chalara* states of *Ceratocystis adiposa* (figure 22), *Ceratocystis paradoxa* (figure 23) and *Thielaviopsis basicola* (figure 24) will now be discussed. The *Chalara* states of *Ceratocystis fimbriata* and *C. radicicola* are similar and are not illustrated. Developmental plasticity (prediction 6) is very evident in all of these species. In particular, free variation between holoblastic and enteroblastic modes is clear in the *Chalara* state of *C. paradoxa*. All of these species have both hyaline and dark conidial stages (prediction 2), and the dark conidial stages tend to have germ slits or pores (prediction 3). In the case of the *Chalara* state of *C. adiposa* the dark conidia appear to lack germ slits or pores, but they are rough walled (prediction 4). All of these species clearly form a related cluster (all known teleomorphs belong in *Ceratocystis* Ellis & Halstead) and within this cluster inter-specific variation can be observed between smooth and rough walled conidia (prediction 5). It might also be noted that in the *Chalara* state of *C. paradoxa* different parts of the hypothetical ancestral life cycle have been lost compared to *C. thielavioides* (prediction 6).

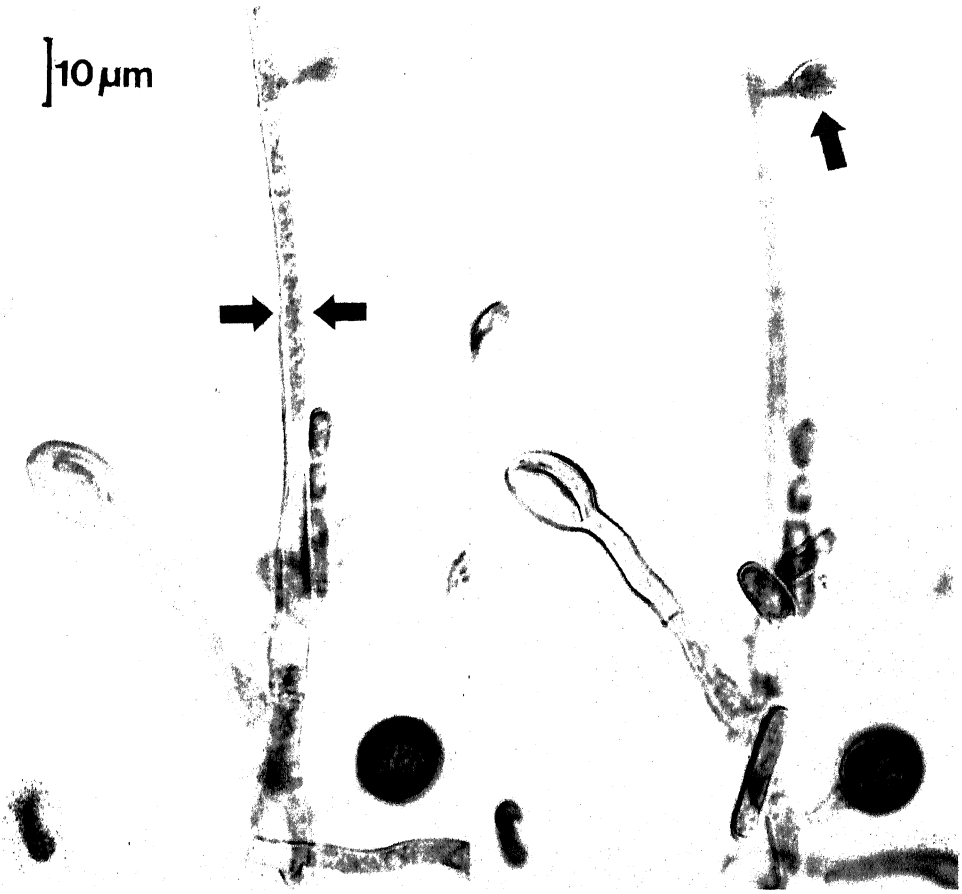


Figure 21. Plasticity of development in *C. thielavioides* resulting in a structure very similar to the basauxic conidiophore of *Arthrinium*: **a.** focused to show the conidiogenous cell and chain of conidia emerging (top of collarette marked with arrows); **b.** refocused to show dark conidial stage (arrow) being produced directly from one of the hyaline conidia.

9.3 *Species of Wardomyces Brooks and Hansford and Gamsia Morelet*

The development of the dark conidium state of *Wardomyces inflatus* is shown semidiagrammatically in figure 25. The conidiogenous cell is hyaline to pale brown and is borne in a more or less well-defined cluster on a more or less well-defined conidiophore or on a vegetative hypha. The conidium is initiated holoblastically by apical wall building, with some synchronous diffuse wall building and, after delimitation with a two-ply septum, rapidly turns brown and becomes rather thicker-walled than the conidiogenous cell which bore it. The mature conidium has a longitudinal germ slit. Each conidiogenous cell may produce only one conidium, or it may proliferate holoblastically and sympodially by a replacement wall building apex after the first conidium has been delimited. A second conidium may then be initiated holoblastically. One conidiogenous cell generally does not produce more than two or three conidia in this way, and the conidia normally secede schizolytically. In other species of *Wardomyces* the development of the dark conidia is similar, except that in

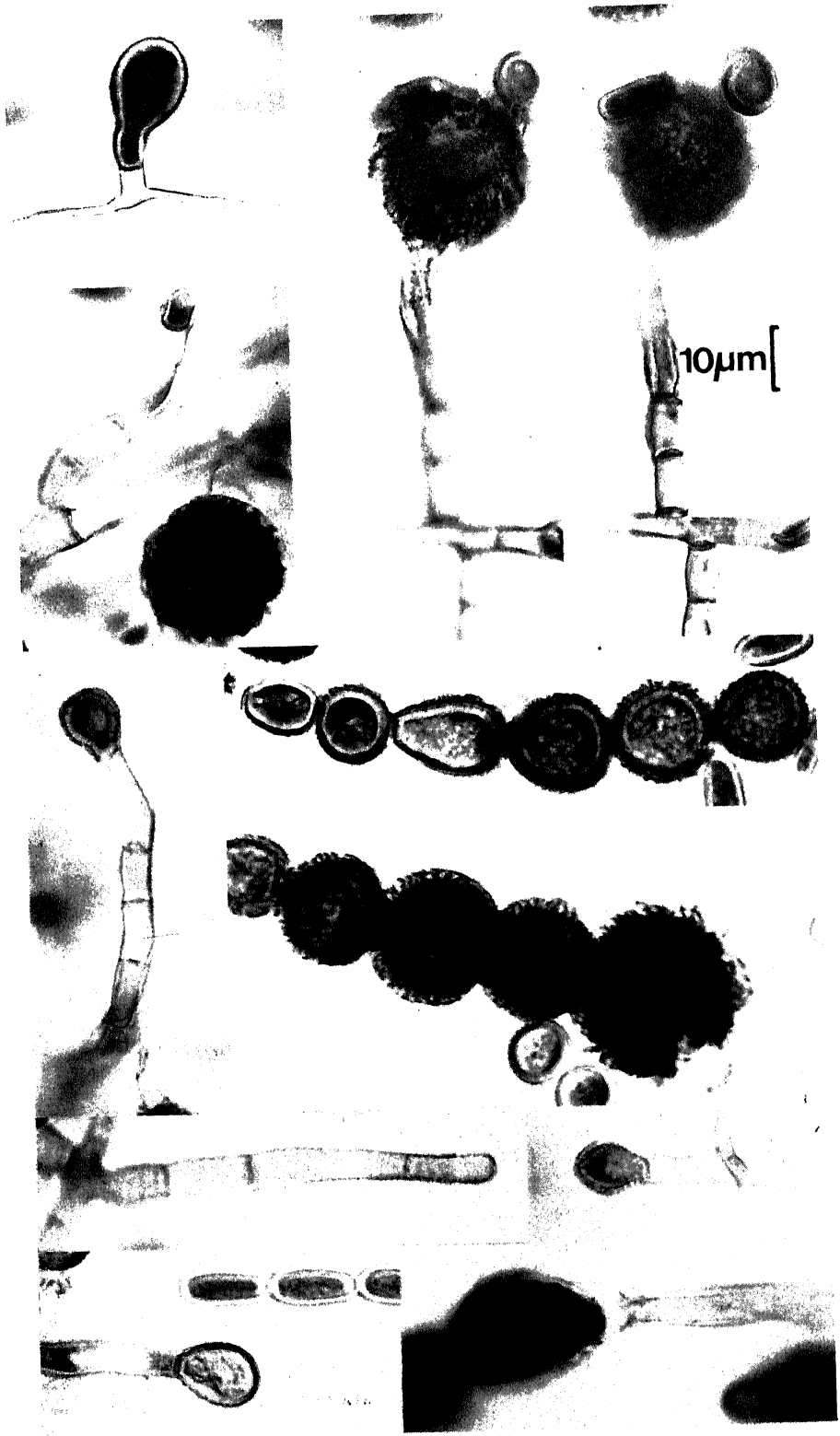


Figure 22. *Chalara* and other anamorphs of *Ceratocystis adiposa*: examples showing developmental plasticity.

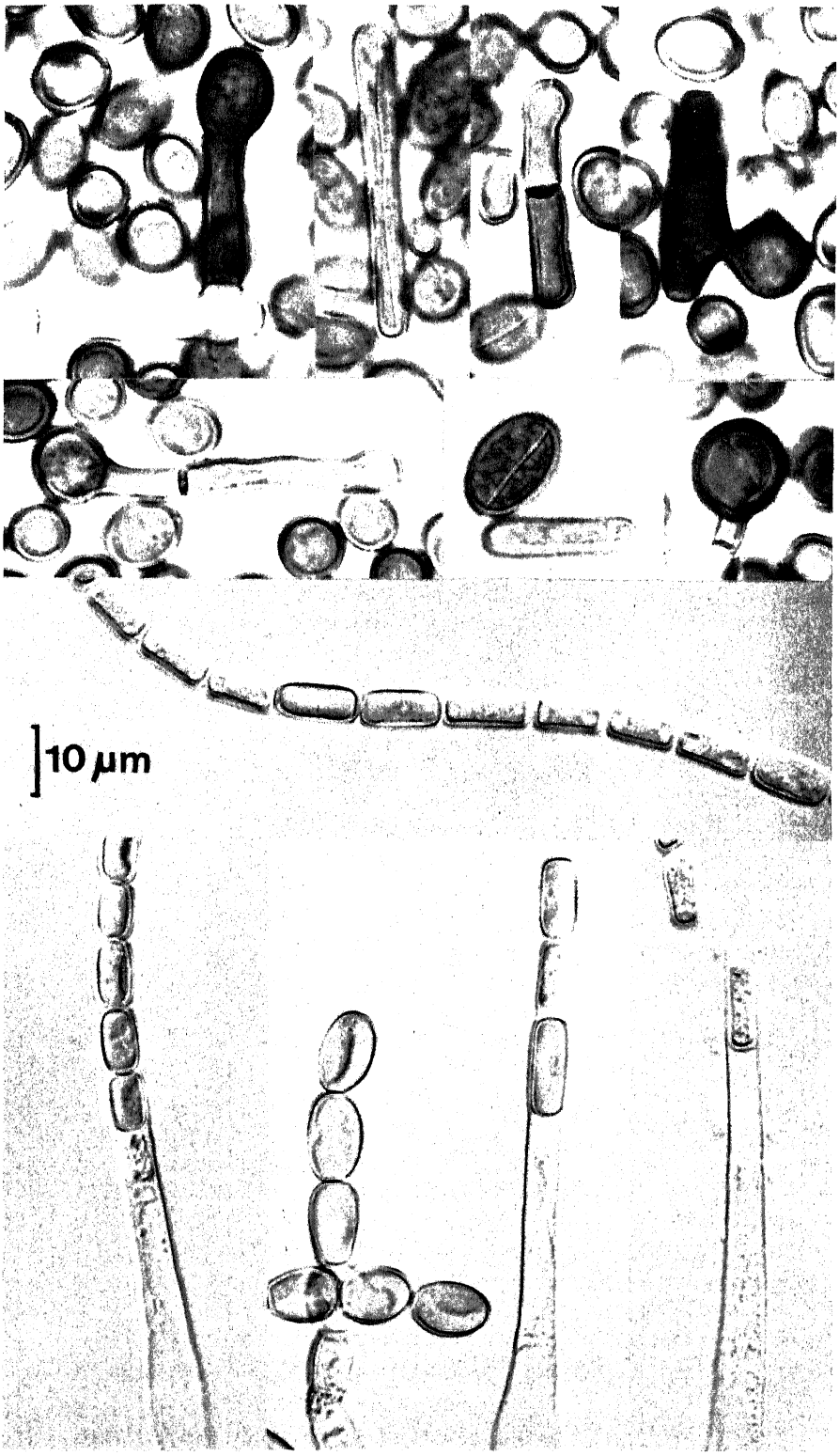


Figure 23. *Chalara paradoxa*: Examples showing developmental plasticity.

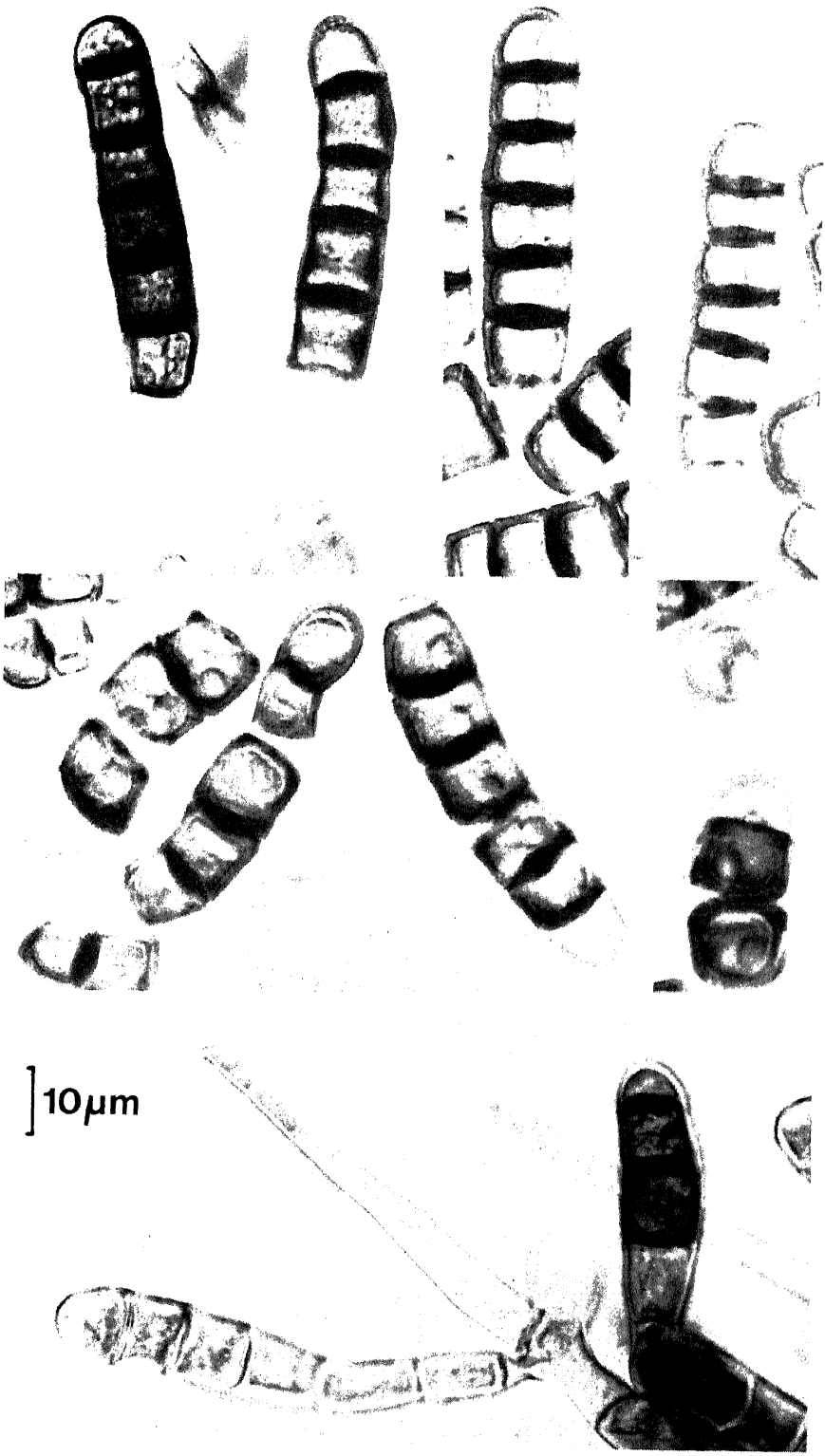
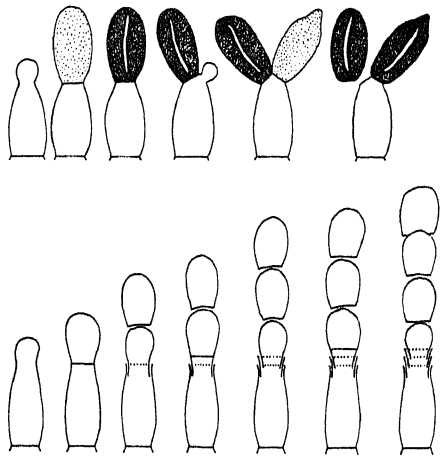


Figure 24. *Thielaviopsis basicola*: examples showing developmental plasticity.



Figures 25, 26. Development of the **25.** dark and **26.** hyaline conidial stages of *Wardomyces inflatus*.

W. humicola each conidiogenous cell produced only one conidium which secedes by rhexolysis, destroying the conidiogenous cell.

The development of the hyaline conidium synanamorph of this species is shown semi-diagrammatically in figure 26. It is a typical member of the genus *Scopulariopsis* Bainier. The conidiogenous cell is hyaline and is borne in a more or less well-defined cluster on a more or less well-defined conidiophore or on a vegetative hypha. The first conidium is initiated holoblastically by apical wall building, with some synchronous diffuse wall building and, after delimitation with a two-ply septum, secedes schizolytically, but remains hyaline. As secession of the first conidium occurs, the conidiogenous cell proliferates enteroblastically and percurrently by a replacement wall building apex, pushing the first conidium away from the point where it was joined to the conidiogenous cell. After the proliferating conidiogenous cell has elongated slightly, a second conidium is initiated holoblastically and the cycle is repeated, eventually producing a false (Subramanian 1972) chain of conidia from an 'annellide'. *Gamsia dimera*, the only member of its genus, is very similar to *Wardomyces* species in development, with a similar *Scopulariopsis*-like synanamorph. It differs principally because the synanamorph has one-septate conidia.

Species of *Wardomyces*/*Scopulariopsis* differ from the hypothetical ancestor of *Arthrinium* species because they do not have the wall building ring form of development at any point in the life cycle. In its place is the rather simpler replacement wall building apex system which, as has previously been observed, must have evolved many times. *Wardomyces*/*Scopulariopsis* species do, however, have both hyaline and dark conidial stages (prediction 2), and the dark conidia have germ slits (prediction 3). If the hypothesis is correct, one might also expect to find species closely related to *Wardomyces* or *Scopulariopsis* but with rough walled dark conidia without germ slits. These may be found in *Echinobotryum atrum* (figure 27), a synnematous species with rough-walled dark conidia without germ slits and a *Scopulariopsis* synanamorph (prediction 4). The difference between rough and smooth dark conidial states in this group seems unlikely to be significant at higher than species level (prediction 5).

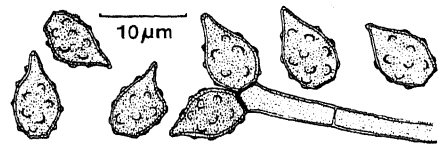


Figure 27. Rough-walled conidia of *Echinobotryum atrum*.

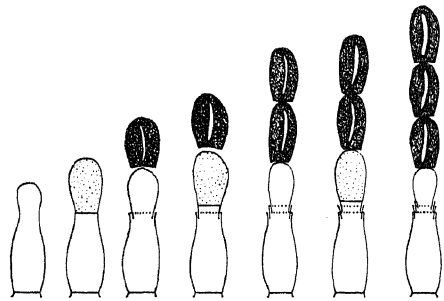


Figure 28. Development of *Wardomycopsis inopinata*.

9.4 *Wardomycopsis inopinata Udagawa & Furuya*

Wardomycopsis inopinata is the type of this monotypic genus. It has not been possible to examine a specimen of this fungus, and it is therefore only known from its original illustration and description (Udagawa and Furuya 1978). Like many species of *Wardomyces* and *Scopulariopsis*, it has a teleomorph in the genus *Microascus* Zukal. Unlike species of *Wardomyces*, *Gamsia* or *Scopulariopsis*, however, it has no known synanamorphs. The development of *W. inopinata*, which is shown semi-diagrammatically (based on the original illustration and description by Udagawa and Furuya 1978) in figure 28, can clearly be seen to be a remarkable combination of a *Scopulariopsis*-like conidiogenous cell producing *Wardomyces*-like conidia by a percurrently proliferating replacement wall building apex system. It is interpreted here as a very clear example of developmental plasticity, with part of the hypothetical ancestral life cycle having been lost (prediction 6) so that the *Scopulariopsis*-like conidiogenous cells do not produce hyaline conidia (which would disperse, germinate, produce a mycelium with conidiogenous cells and then a dark conidium synanamorph), but instead go straight on to produce the *Wardomyces*-like conidium. If this is correct then it would be hard to imagine a more splendid example of a shortening in conidial production cycles than is found in *W. inopinata*!

9.5 *Mammaria echinobotryoides*

The development of this species is shown semi-diagrammatically in figure 29. This species is developmentally very plastic. Some strains may produce minute hyaline globose conidia by a replacement wall building apex system, reminiscent of the *Scopulariopsis* synanamorphs of *Wardomyces* species, but different because the conidiogenous cell does not elongate with each proliferation (*i.e.* it is a 'phialide' not an 'annellide') (figure 29a). A dark conidial state with germ slits is always produced, and this is similar to many species of *Wardomyces* (figure 29b). The fungus can also

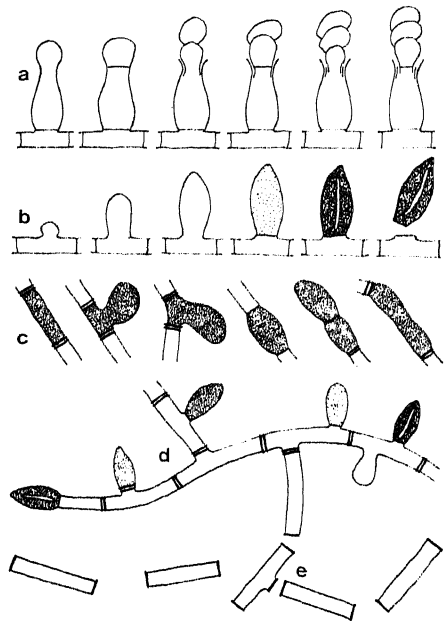


Figure 29. Development of *Mammaria echinobotryoides*.

produce dark conidia intercalary on vegetative hyphae, and these may take a variety of shapes and sizes (figure 29c). Most significant of all, however, is the presence of darkened septa on the hyphae bearing these dark conidia (figure 29d). Such hyphae are branched (prediction 7) and not infrequently fragment at the darkened septa to form 'arthroconidia' (figure 29e) (prediction 8).

10. Assessment of the hypothesis

The hypothesis makes one main assumption: that species of *Arthrinium* have evolved from an ancestor producing two conidial stages, one hyaline and the other dark. Some other minor assumptions are also made: that the ancestor also had a wall building ring stage and that if two conidial stages evolved to be adjacent one might well lose its dispersal function. Given those assumptions, the hypothesis predicts developmental plasticity in species of *Arthrinium* and related fungi: a phenomenon which has already been observed in pure cultures. The hypothesis also provides a possible explanation of the two different types of 'basauxic conidiophores' in *Arthrinium*, and makes clear the reason for the similarity between the 'conidiophore mother cell' and wall building ring 'phialides'. Indeed the differences between these two organs suddenly seems very insignificant!

By comparison, the conventional classification systems based on assigning individual species to rather arbitrary developmental categories (Hughes 1953; Kendrick 1971; Ellis 1971, 1976) do not predict developmental plasticity, nor can they explain why *Arthrinium* species have two types of 'basauxic conidiophores', nor can they relate the 'conidiophore mother cell' to the very similar wall building ring 'phialides'. The conventional classification systems encourage the observer of these fungi to reject the

developmental plasticity observed in their cultures as somehow 'abnormal'. If the hypothesis proposed here is followed, however, it becomes possible to regard the same developmental plasticity as a fascinating 'window' looking in on genetic capability which would otherwise remain locked up and invisible.

Nine predictions from the hypothesis were made about fungi likely to be similar to species of *Arthrinium*. Examples were cited and discussed which fulfilled each of these predictions. Although all of the fungi used as examples would have been regarded under conventional classifications as totally unrelated to *Arthrinium* (or, indeed, in many cases to each other), it became clear as they were analysed that their patterns of development showed certain real points of affinity with those of *Arthrinium* species. It can therefore be concluded that development in the *Arthrinium* group is not fundamentally different from that of other deuteromycetes, and that Hughes' (1953) system makes these fungi appear more different than they really are. In the following paragraphs some comments are made about the possible taxonomic affinities between the *Arthrinium* group and selected other deuteromycetes.

A cluster of genera centred on *Arthrinium* can readily be identified. All produce dark conidia with germ slits, sometimes also germ pores and all apparently lack a hyaline conidial synanamorph. Most produce a specialised fertile hypha from a wall building ring structure apparently derived from an ancestral 'phialide'. *Papularia* may be used for *Arthrinium*-like species with narrow specialised hyphae, but the distinction seems unlikely to correlate with generic differences in any teleomorph. Many species of *Nigrospora* belong here, being simply *Arthrinium*/*Papularia* species with an even more truncated life cycle. Some of these species of *Nigrospora* show specialised adaptations to enable their conidia to be discharged violently.

Species of *Cordella, Pteroconium* and *Endocalyx* also belong here, these genera showing minor adaptations (a sporodochial habit in the first, setae in the second and a cupulate habit in the third) again not likely to correlate with major generic differences in teleomorphs. *Dictyoarthrinium* fits in this group too: although its conidia are ultimately multi-septate, they are indistinguishable in their early stages of development from those of *Arthrinium*, and many other features also correlate well with this group. It is predicted that any teleomorphs found for these fungi will occur in and around *Apiospora*.

Species of *Spegazzinia*, however, are excluded from this group even though they have traditionally been classified close to *Arthrinium*. Their conidia remain hyaline until after they become septate (contrast with *Dictyoarthrinium*), the specialised hypha never produces more than one conidium, and is itself usually rather pigmented. These and other characteristics suggest that members of this genus are not closely related to *Arthrinium*.

Another cluster of genera may be recognised around certain species of *Chalara*. These *Chalara* species tend to be anamorphs of *Ceratocystis* and all have conidiogenous cells of the hyaline conidial state with a rather poorly defined transition between venter and collarette. They show considerable developmental plasticity, being able to produce their dark conidial stage in a wide variety of manners. As a result, several genera have been described in the past to accommodate this variability, for example, *Chalaropsis* Peyronel and *Thielaviopsis* Went. Members of this cluster show some relation to the *Arthrinium* cluster, since they both have ring wall building systems and an ability to produce dark conidia with germ pores or slits. The *Chalara* cluster has, however, retained the ability to produce both hyaline and dark conidia suggesting that

teleomorphs of the two clusters would probably not be found in the same ascomycete family.

A third cluster of genera centres around *Wardomyces* and includes *Cephalotrichum* Link, *Echinobotryum* Corda, *Gamsia*, *Scopulariopsis*, *Trichurus* Clements and *Wardomycopsis*. Teleomorphs occur in and around *Microascus*. Like the *Chalara* cluster, these fungi mostly produce both hyaline and dark conidial states and show the same sort of variation between smooth and rough dark conidia, with or without germ pores or slits. They have marked developmental plasticity and a facility for shortening of life cycles as seen in *Wardomycopsis*. Unlike the *Chalara* or *Arthrinium* cluster, however, they have no wall building ring mode, suggesting that they are more distantly related to the *Arthrinium* cluster than is the *Chalara* cluster.

Some species of *Mammaria* Cesati, *Gilmaniella* Barron and *Humicola* Traaen may also belong in this cluster. *Mammaria echinobotryoides* is so developmentally plastic that it could be placed almost anywhere among these fungi. Characteristics found in most isolates suggest it occupies a position intermediate between the *Arthrinium* and the *Wardomyces* cluster. It is possible that different isolates identified as *Mammaria echinobotryoides* will be found after more detailed study to relate to more than one species. Species of *Gilmaniella* and *Humicola* are also rather difficult to place with confidence, since they too show a degree of developmental plasticity. It would, perhaps, not be surprising to find that different species within these two genera belong in phylogenetically rather diverse groups.

In the time available to carry out this study it was not possible to examine every hyphomycete potentially related to the *Arthrinium* group in equal detail. Some genera examined only superficially include *Catenularia* Grove, *Conioscypha* Höhnel, *Megalodochium* Deighton, *Paathramaya* Subramanian and *Sporoschisma* Berkeley & Broome. Not all hyphomycetes producing dark conidia with germ slits or pores are, however, necessarily so closely related to the *Arthrinium* group. Species of *Conoplea* Persoon, for example, differ in many respects of form and development from the *Arthrinium* group. Almost the only features they have in common are the dark conidia with germ slits or pores. That they should be so different is not very surprising, since teleomorphs of *Conoplea* are known only from the Pezizales. Species of *Melanographium* Saccardo too appear to have little in common with the *Arthrinium* group apart from their dark conidia with germ slits. It must therefore be concluded that dark conidia with germ slits or pores have evolved more than once, and that the same caution should be used when applying this as with any other characteristic.

11. Discussion

The hypothesis offered in this paper allows individual fungi within a cluster to be related to each other in a manner not possible under the systems of Saccardo (1886) or Hughes (1953). In the system of Saccardo (1886) hyphomycetes were classified according to conidial pigmentation, shape and septation. The effect of this was to separate *Dictyoarthrinium*, for example, from *Arthrinium*, because the one has septate conidia and the other does not, or the *Thielaviopsis* anamorph of *Ceratocystis paradoxa* from its *Chalara* synanamorph, because conidia are brown in the one and hyaline in the other. The same system also separated species of *Wardomyces* from their *Scopulariopsis* synanamorphs on the grounds of conidial pigmentation, and was not

able to explain the relationship between species of *Wardomyces*, *Scopulariopsis* and *Wardomycopsis*.

In the system of Hughes (1953) hyphomycetes were assigned to one of a small number of sections "based primarily upon the different types of conidiophore and conidium development". The effect of this was to separate *Arthrinium* from *Nigrospora*, because the one has 'basauxic conidiophores' and the other does not, or the *Thielaviopsis* anamorph of *Ceratocystis paradoxa* from its *Chalara* synanamorph, because development in the one is 'thallic' and in the other it is 'phialidic'. Species of *Wardomyces* were also separated under this system from their *Scopulariopsis* synanamorphs, because the former produced their conidia holoblastically and the latter enteroblastically.

If the present hypothesis is followed, however, species of *Arthrinium* and *Nigrospora*, for example, can be regarded as closely related in spite of their differences in development; so can species of *Wardomyces* and *Scopulariopsis* in spite of their differences in conidial pigmentation. This is of interest because a system of this nature may parallel more closely the classification of teleomorphs of these fungi: teleomorphs of species within each cluster belong to the same or similar genera and the differences in teleomorphs between clusters is of about the level one might expect from the differences in anamorphs between clusters.

Analysis of the various developmental characteristics exhibited by these and other hyphomycetes showed that many of the patterns of development currently regarded as significant could have evolved many times. One would therefore expect fungi producing conidia by replacement wall building apex systems, for example, to turn up in widely diverse phylogenetic groups. This is, of course, exactly what happens: many species of *Ceratocystis* do not have *Chalara* anamorphs. Instead one finds anamorphs in genera such as *Graphilbum* Upadhyay & Kendrick, *Graphium* Corda, *Leptographium* Lagerberg & Melin, *Pesotum* Crane & Schoknecht, *Phialocephala* Kendrick, *Phialographium* Upadhyay & Kendrick, *Sporothrix* Hectoen & Perkins and *Verticicladiella* Hughes. It has been speculated that the genus *Ceratocystis* is polyphyletic, its simple perithecia being the result in different species of convergent evolution (Samuels and Müller 1979). The view of anamorphic classification proposed here neither supports nor argues against this speculation: both possibilities can be accommodated because so many of the anamorphs are simple replacement wall building apex system fungi which the present hypothesis predicts would occur throughout the ascomycetes. It follows that much caution is needed in using anamorph data to support splits in teleomorph taxa, and simplistic interpretations should be avoided.

Some idea of the ease with which conidiogenous structures of this sort can evolve may be gained by remembering that inoperculate discomycetes of the family Hyaloscyphaceae also have anamorphs in both *Chalara* and *Haplographium* Berkeley & Broome, a genus very similar to *Verticicladiella*. *Sageria* Funk, another genus of the inoperculate discomycetes, has anamorphs in *Ascoconidium* Seaver, a hyphomycete very similar in development to *Chalara*. Accordingly, *Chalara* (with its satellite genera such as *Chaetochalara* B. Sutton & Pirozynski) and members of the *Leptographium* complex of genera are interpreted here as being rife with convergent evolution. The same may be said about *Bloxamia* Berkeley & Broome, which at present contains species exhibiting a wide variety of developmental cocktails. *Bloxamia leucophthalma* has a wall building ring (Glawe 1984) and minimal narrowing between venter and

collarette; *B. bohemica* is like many of the smaller *Chalara* species, only sporodochial, and *B. sanctae-insulae* produces conidia by a replacement wall building apex system. *Phialocephala* contains both replacement wall building apex 'phialides' (possibly closely related to species of *Leptographium*) and wall building ring 'phialides' (possibly close to *Chalara* species), and as such is also in need of critical revision.

One of the interesting features of all of these fungi whom became apparent as they were analysed was the facility with which they were apparently able to switch on or off the genes needed to produce particular stages. Thus, for example, the ability to produce a dark conidium with a germ slit is present in both *Wardomyces* species and *Wardomycopsis inopinata*. Because they all have teleomorphs in the same ascomycete genus, it is clear they are closely related. In species of *Wardomyces*, however, the genes for a dark conidium are activated at a totally different stage from that in *W. inopinata*. This raises the interesting question of whether such genes can only be activated when these fungi are in their anamorphic condition, or whether in closely related fungi a similar set of genes could be in action producing dark ascospores with germ slits. This question appears to have received little consideration in the past, but if it is a genuine possibility, the implications for ascomycete and deuteromycete taxonomy could be enormous.

Acknowledgements

Drs P F Cannon, P M Kirk and B C Sutton and Prof. D L Hawksworth are thanked for valuable discussions. Mrs M Rainbow is thanked for technical assistance. The author also thanks his wife, Helen, for her perceptive comments on the manuscripts.

References

Arx J A von 1974 *The genera of fungi sporulating in pure culture* (Vaduz; J Cramer)

Cole G T 1983 *Graphiola phoenicis*: a taxonomic enigma; *Mycologia* **75** 93–116

Cole G T and Samson R A 1979 *Patterns of development in conidial fungi* (London, San Francisco, Melbourne: Pitman)

Cooke W B 1954 The genus *Arthrinium; Mycologia* **46** 815–822

Ellis M B 1971 *Dematiaceous hyphomycetes* (Kew: Commonwealth Mycological Institute)

Ellis M B 1976 *More dematiaceous hyphomycetes* (Kew: Commonwealth Mycological Institute)

Ellis M B, Ellis E A and Ellis J P 1951 British marsh and fen fungi II; *Trans. Br. Mycol. Soc.* **34** 497–514

Glawe D A 1984 *Bloxamia truncata* in artificial culture; *Mycologia* **76** 741–745

Gooday G W 1984 The hyphal tip in *Fungal differentiation, a contemporary synthesis* (ed.) J E Smith (New York and Basel; Marcel Dekker Inc.)

Hawksworth D L, Sutton B C and Ainsworth G C 1983 *Ainsworth & Bisby's dictionary of the fungi* (Kew: Commonwealth Mycological Institute)

Hudson H J 1963a Pyrenomycetes of sugar cane and other grasses in Jamaica; *Trans. Br. Mycol. Soc.* **46** 19–23

Hudson H J 1963b The perfect state of *Nigrospora oryzae; Trans. Br. Mycol. Soc.* **46** 355–360

Hughes S J 1953 Conidiophores, conidia and classification; *Can. J. Bot.* **31** 577–659

Kendrick W B 1971 *Taxonomy of fungi imperfecti*; (Toronto and Buffalo: University of Toronto Press)

Kendrick W B and DiCosmo F 1979 Teleomorph-anamorph connections in ascomycetes in *The whole fungus* (ed.) Bryce Kendrick (National Museum of Natural Sciences, National Museums of Canada and The Kananaskis Foundation)

Mason E W 1933 Annotated account of fungi received at the Imperial Mycological Institute; *Mycol. Pap.* **3** (Kew: Commonwealth Mycological Institute)

Matsushima T 1975 *Icones microfungorum a Matsushima lectorum*; (Kobe: T Matsushima)

Minter D W 1984 New concepts in the interpretation of conidiogenesis in deuteromycetes; *Microbiol. Sci.* **1** 86–89

Minter D W, Kirk P M and Sutton B C 1982 Holoblastic phialides; *Trans. Br. Mycol. Soc.* **79** 75–93

Minter D W, Kirk P M and Sutton B C 1983a Thallic phialides; *Trans. Br. Mycol. Soc.* **80** 39–66

Minter D W, Sutton B C and Brady B L 1983b What are phialides anyway; *Trans. Br. Mycol. Soc.* **81** 109–120

Müller E and Arx J A von 1962 Die gattungen der didymosporen pyrenomyceten; *Beitr. Kryptogflora Schweiz* **11** 1–922

Popper K 1959 *The logic of scientific discovery*, (London: Hutchinson)

Okada G and Tubaki K 1984 A new species and a new variety of *Endocalyx* (Deuteromycotina) from Japan; *Mycologia* **76** 300–313

Saccardo P A 1886 *Sylloge fungorum* **4** (Pavia: P A Saccardo)

Samuels G J and Müller E 1979 Life-history studies of Brazilian ascomycetes; *Sydowia* **31** 169–179

Subramanian C V 1972 Conidial chains, their nature and significance in the taxonomy of Hyphomycetes; *Curr. Sci.* **41** 43–49

Subramanian C V 1979 Phialidic hyphomycetes and their teleomorphs—an analysis in *The whole fungus* (ed.) Bryce Kendrick (National Museum of Natural Sciences, National Museums of Canada and The Kananaskis Foundation)

Sutton B C 1980 *The coelomycetes*; (Kew: Commonwealth Mycological Institute)

Tubaki K 1958 Studies on the Japanese hyphomycetes. V. Leaf and stem group with a discussion of the classification of hyphomycetes and their perfect stages; *J. Hattori Bot. Lab.* **20** 142–244

Udagawa S and Furuya K 1978 A new species of *Microascus* and its peculiar conidial state; *Mycotaxon* **7** 91–96

Webster J 1952 Spore projection in the hyphomycete *Nigrospora sphaerica; New Phytol.* **51** 229–235

Webster J 1966 Spore projection in *Epicoccum* and *Arthrinium; Trans. Br. Mycol. Soc.* **49** 339–343

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 309–317.
© Printed in India.

New and rare entomogenous fungi from Amazonia

ROBERT A SAMSON and HARRY C EVANS*

Centraalbureau voor Schimmelcultures, P.O. Box 273, 3740 AG Baarn, the Netherlands

* Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF, UK

Abstract. A new sclerotium-producing species on an ant host, *Hirsutella subramanianii*, and a new *Cordyceps* species, *C. cantharelloides*, with agaric-like fruitbodies on a wood-boring coleopteran larva are described and illustrated from specimens collected in the Amazonian rain forests.

Keywords. Entomogenous fungi; *Hirsutella subramanianii; Cordyceps cantharelloides.*

1. Introduction

Entomogenous fungi have been collected by the junior author, over a 6-year period from 1974 to 1980, in the tropical forest of the Ecuadorian upper Amazon and the Brazilian lower Amazon, a region commonly known as Amazonia. These forests possess a rich and varied entomogenous mycoflora and some of the *Cordyceps* species from ant hosts have been the subject of recent papers (Evans and Samson 1982, 1984).

In this contribution two new and apparently rare species from Amazonia are described. Despite the relative paucity of material, formal descriptions of these fungi are considered justified, because of their uniqueness and unusual morphology and hence their taxonomic significance. Unfortunately repeated attempts to isolate these fungi on agar media failed. We name the *Hirsutella* species after Prof. C V Subramanian as a sign of appreciation for his many contributions to hyphomycete taxonomy.

2. *Hirsutella subramanianii* sp. nov. (figures 1–2)

Mycelium internum e catenis corporum hyphalium oblongorum vel rectangularium, hyalinorum, tenuitunicatorum, (6·5–)8·5–11(–13) × 5·5–7 μm, compositum. Mycelium externum inter segmenta corporis protrudens, primum album, floccosum, hyphis hyalinis, levibus, septatis, 2–3 μm latis compositum, deinde brunnescens et fasciculatum. Phialides laterales sparsae in hyphis aeriis singulis, hyalinae, leves, subcylindricae, 15–25 μm longae, deorsum ad 2·5–3·5 μm inflatae, sursum in collulum longum, verrucosum, 0·6–1·2 μm latum, angustatae. Conidia hyalina, obclavata vel curvata (forma musae fructuum), continua, 10–3·5 × 1·8–2·5 μm, strato mucido tenui circumdata. Sclerotia copiosa in mycelio externo, primum albae, maturitate brunnescentia, verrucosa, globosa, 70–95 μm diam, e cellulis pseudocollenchymaticis globosis vel irregularibus crassitunicatis composita.

Holotypus IMI 290522, ad *Pachycondylam* sp. (Ponerinae, Formicidae), in terra forestali lectam, Monte Dourado, Rio Jari, Pará in Brasilia, H C Evans, 19 Jan. 1980.

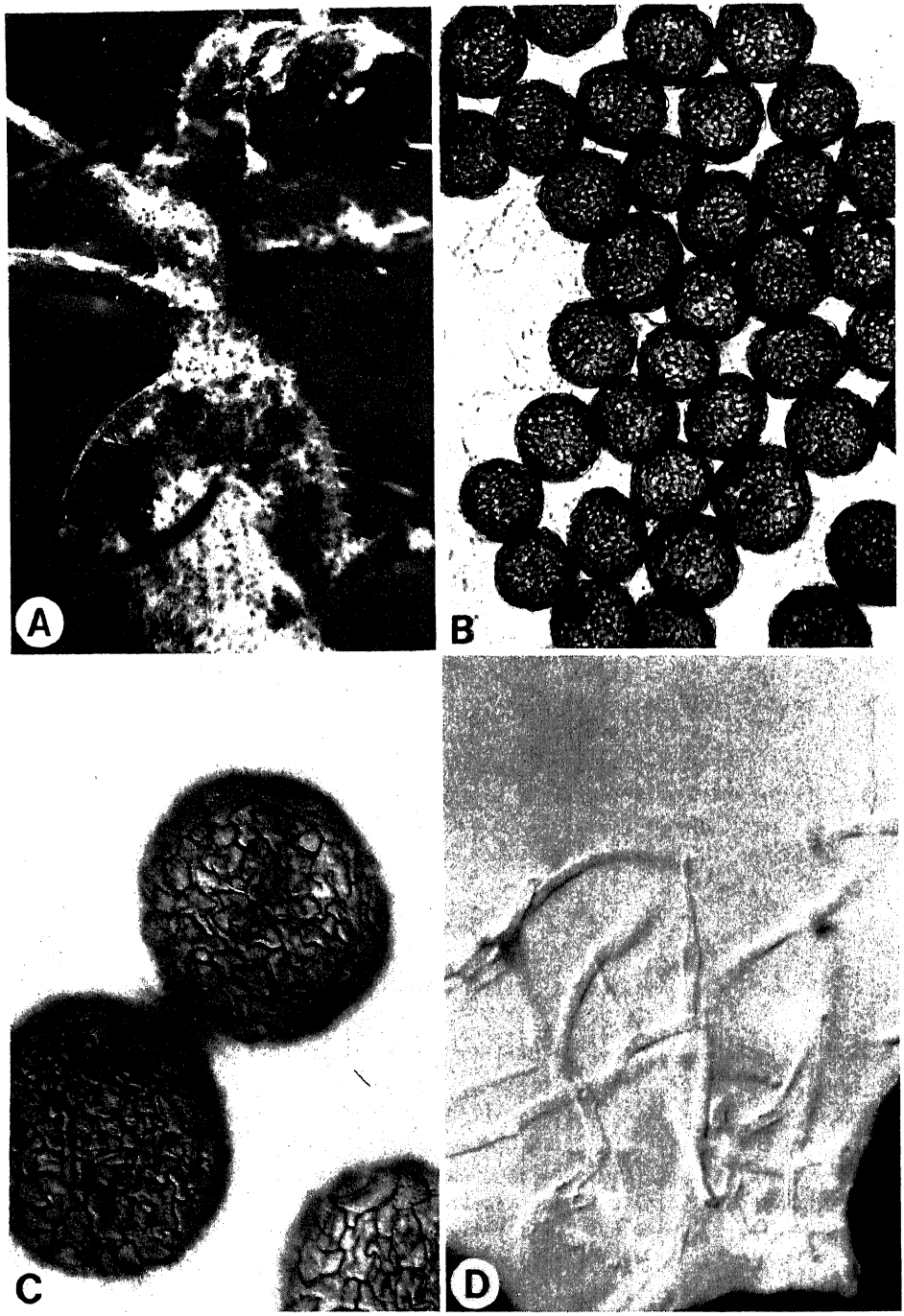


Figure 1. *Hirsutella subramanianii*. **A.** Ponerine ant showing floccose, external mycelium and dark-coloured sclerotia (× 1·5). **B–C.** Sclerotia. Note the irregular dark-walled outer cells (B. × 190, C. × 550) **D.** Phialides (× 950).

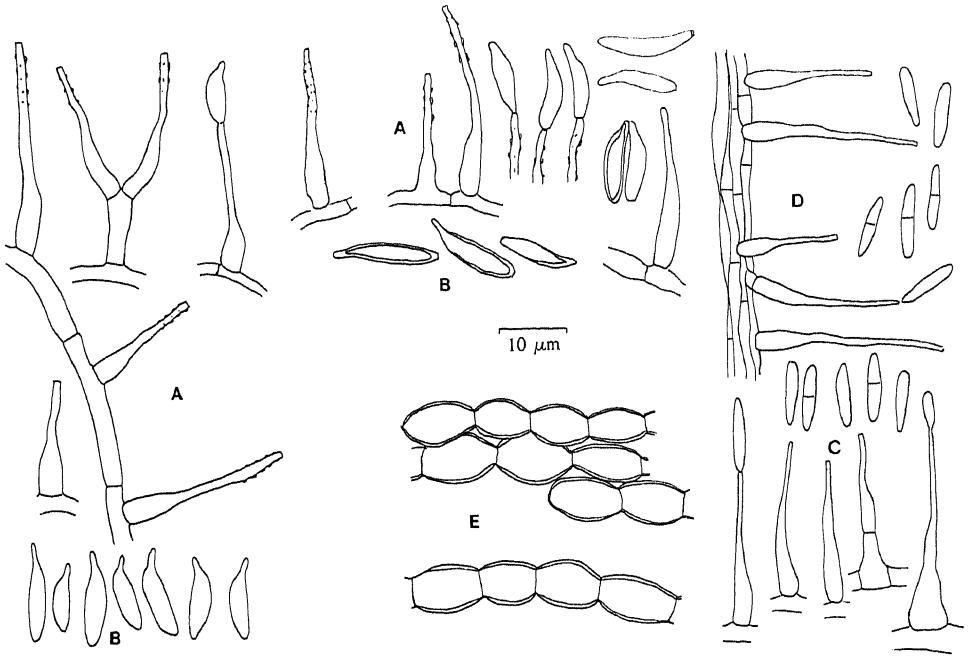


Figure 2. *Hirsutella subramanianii.* Camera lucida drawings. **A–B.** A-phialides with conidia **C.** B-phialides and conidia **D.** B-phialides on synnema. **E.** hyphal bodies.

Internal mycelium consisting of compact chains of oblong to rectangular, hyaline, thin-walled hyphal bodies, (6·5–)8·5–11 (–13) × 5·5–7 μm. *External mycelium* arising from body joints, initially white, floccose; hyphae hyaline, smooth, septate, 2–3 μm wide, becoming ginger-brown and uniting into ropes. *Phialides* borne directly on aerial mycelium (mononematous), as scattered lateral outgrowths; hyaline, smooth, subcylindrical, 15–25 μm in length, swollen at the base (2·5–3·5 μm width) tapering gradually to a long, verruculose neck region (0·6–1·2 μm wide). *Conidia* hyaline, curved, obclavate (or banana-shaped), aseptate, 10–13·5 × 1·8–2·5 μm, encased in a thin mucous sheath, giving the conidia a shape with an apical projection and a truncate base. *Sclerotia* formed abundantly on the external mycelium, white at first, when mature chestnut to dark brown, verrucose, globose, 70–95 μm diam, composed of globose to irregular, thick-walled pseudocollenchyma.

Holotype on *Pachycondyla* sp. (Ponerinae, Formicidae), forest soil, Monte Dourado, Rio Jari, Pará, Brazil, 19 Jan. 1980, H C Evans (IMI 290522). *Paratype* on a similar host from the same locality in Herb. CSB, RS 1545.

The paratype is an older specimen, the sclerotia being predominantly dark brown and the external mycelium ginger brown. A narrow cylindrical *synnema*, 11 × 0·2 mm, emerges laterally from the thorax; it is ginger brown at the base, becoming pale yellow or cream towards the fertile apex. Phialides are scattered or locally aggregated, variable but usually with a swollen, up to 5 μm wide, often verrucose basal cell, and a narrow, smooth neck. Conidia hyaline, smooth-walled, 1-septate, narrowly clavate, 8·5–10·5 × 1·2–1·5 μm, without a mucous sheath.

It is considered, therefore, that *Hirsutella subramanianii* may produce two distinct

types of phialides and conidia, as described for *H. stilbelliformis* Evans and Samson from various ant hosts (Evans and Samson 1982). Indeed, *H. subramanianii* helps to clarify some of the complex relationships within the genus *Hirsutella* and supports the conclusions reached by Evans and Samson (1982) when they placed the genus *Synnematium* Speare in synonymy with *Hirsutella*. The type species of that genus, *Synnematium jonesii* Speare, has subulate phialides and produces the conidia in mucoid spore balls, being further characterised by the formation of brown sclerotia. Speare (1920) depicted the conidia in groups and also encased them in individual mucous sheaths, as observed in the holotype of *H. subramanianii*, comparable to the B-conidia of *H. stilbelliformis*. In analogy with this interpretation, the conidiogenous structures occurring on the synnema of the paratype of *H. subramanianii* would correspond to the A-phialides and A-conidia; the latter in both species being typically 1-septate and devoid of a mucous covering.

In conclusion, *H. subramanianii*, with its two types of phialide and conidia and its sclerotia, forms a link between *H. jonesii* (Speare) Evans and Samson, occurring on the homopteran host and a complex series of *Hirsutella* species occurring on ants (Evans and Samson 1982, 1984). By producing two morphologically distinct anamorphs, the fungus may have adapted both to wind (A-conidia) and rainsplash (B-conidia) dispersal. An added function of the mucous sheath, which characteristically darkens with age, is probably to increase conidial longevity by conferring protection from desiccation and solar radiation. The sclerotia are also obviously survival structures possibly enabling *H. subramanianii* to persist in the soil after the ant host has disintegrated.

3. *Cordyceps cantharelloides* sp. nov. (figures 3–5)

Mycelium internum hyalinum, ex hyphis densis, regulariter septatis, 2·5–3 μm latis compositum, corpora hyphalia pauca, globosa ad ovoidea, 14–25 × 12–15 μm. Mycelium externum exiguum, in parvis floccis hospitem obtegens, dilute brunneum, ex hyphis septatis, 1·5–2 μm latis compositum. Stromata bina, simplicia, e parte anteriore hospitis oriunda; stipes fere cylindricus, luteo-brunneus, levis (siccus sulcatus), 15–18 × 2–4mm; pars fertilis terminalis, lateritia, plus minusve discoidea, Cantharelli similis, 10–11 × 3–5mm. Paries externa e cellulis cylindricis palisadiformibus compositum. Perithecia omnino submersa, dense aggregata, papillis sursum protrudentia, anguste obclavata, 260–380 × 110–140 μm, collo longo periphysibus repleto. Asci cylindrici, 140–160 × 4–5 μm, sursum inspissati, in medio inflati et deorsum in stipitem longum angustati. Ascoporae hyalinae, filiformes, multiseptatae, guttulatae, utrinque apiculatae, non secedentes, 75–85 × 2–2·5 μm. Anamorphosis ignota.

Holotypus IMI 290521, ad larvam Coleopterorum, in arbore sylvestri, Palora, Pastaza in Ecuadore, H C Evans, 30 Nov. 1975.

Internal mycelium hyaline, consisting of a dense weft of regularly septated hyphae, 2·5–3 μm wide; hyphal bodies scarce, globose to ovoid, 14–25 × 12–15 μm. *External mycelium* almost lacking on the cadaver, only present on localized spots and then densely covering the host, pale brown, hyphae septate, 1·5–2 μm wide. Stromata paired, unbranched, emerging from the head-region; stalk region yellow-brown, more or less cylindrical, smooth when fresh, but furrowed when dried, 15–18 × 2–4mm; fertile area brick-red when dry, terminal, more or less discoid, strikingly reminiscent of

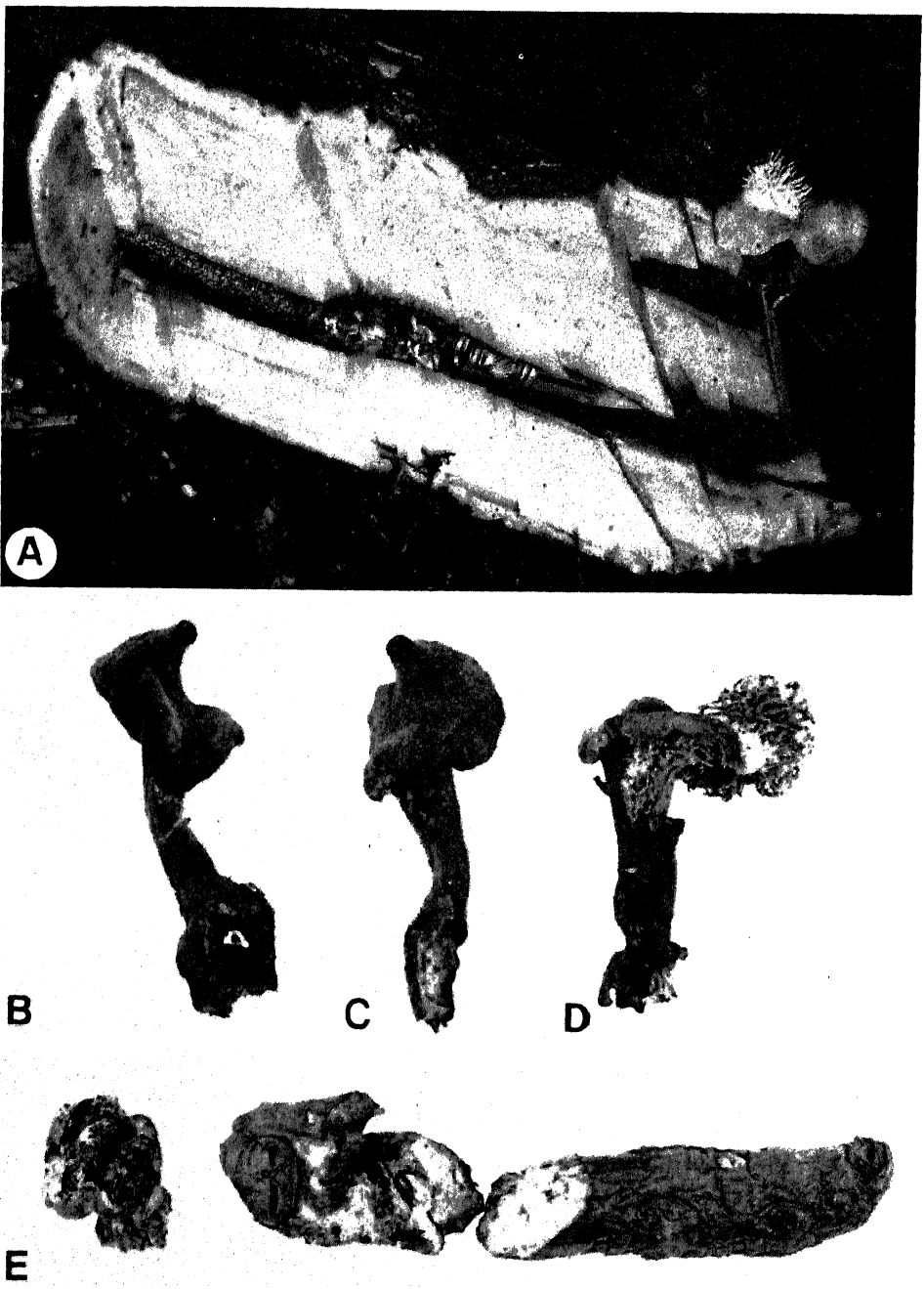


Figure 3. *Cordyceps cantharelloides*. **A.** Clavae *in situ*. Note the fragments of the host and a pencil which has been placed in the tunnel in the living wood, and the two stromata one heavily parasitised, emerging laterally (×1). **B–D.** Dried clavae. **B–C.** viewed from different angles (×2) **D.** Stilbella parasitised clava (×2) **E.** Fragments of large larva of woodboring beetle after extraction (×2).

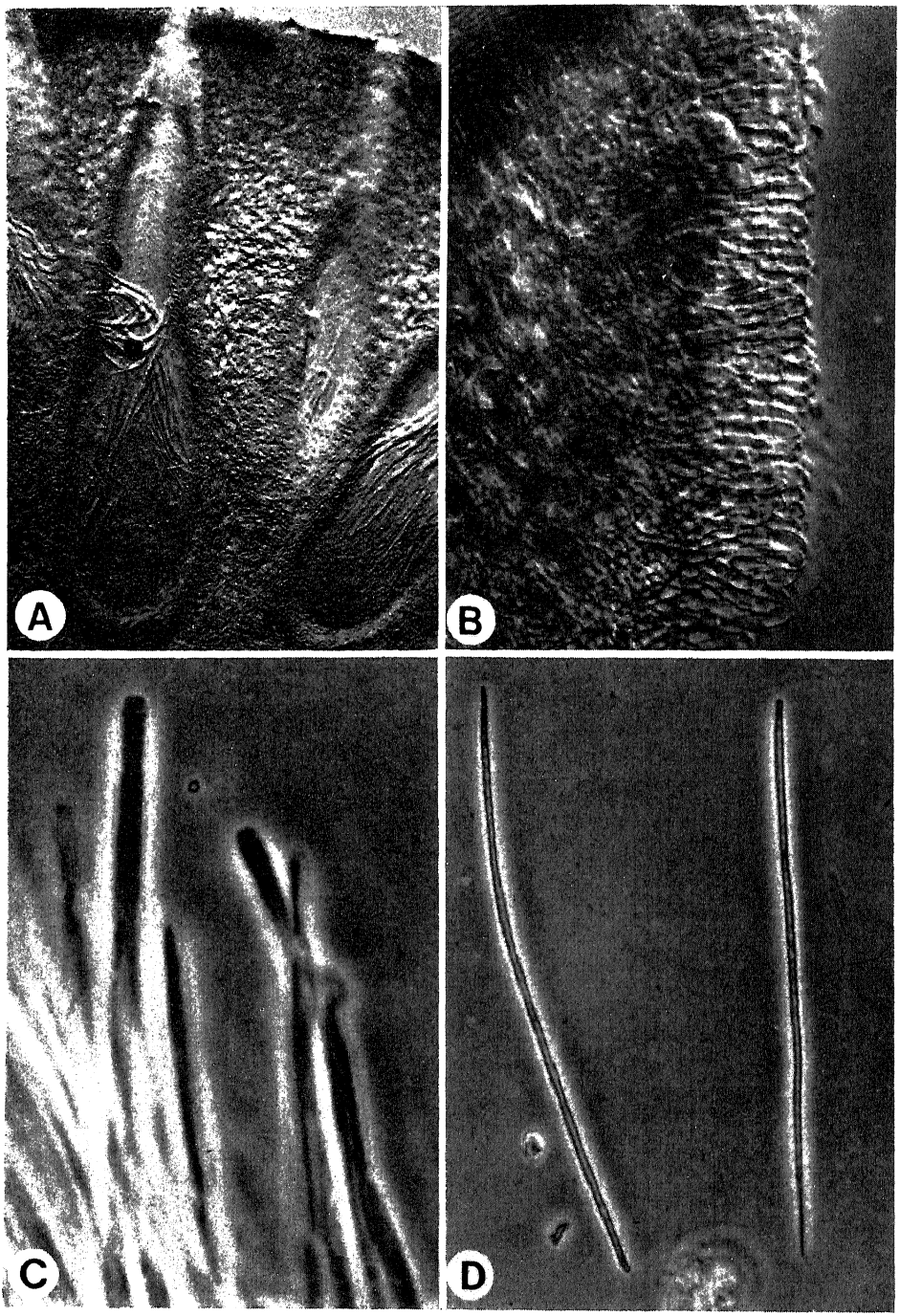


Figure 4. *Cordyceps cantharelloides.* **A.** Perithecia (× 55), **B.** Outer cell wall of fertile head (× 140). **C.** Asci (× 750). **D.** Ascospores (× 550).

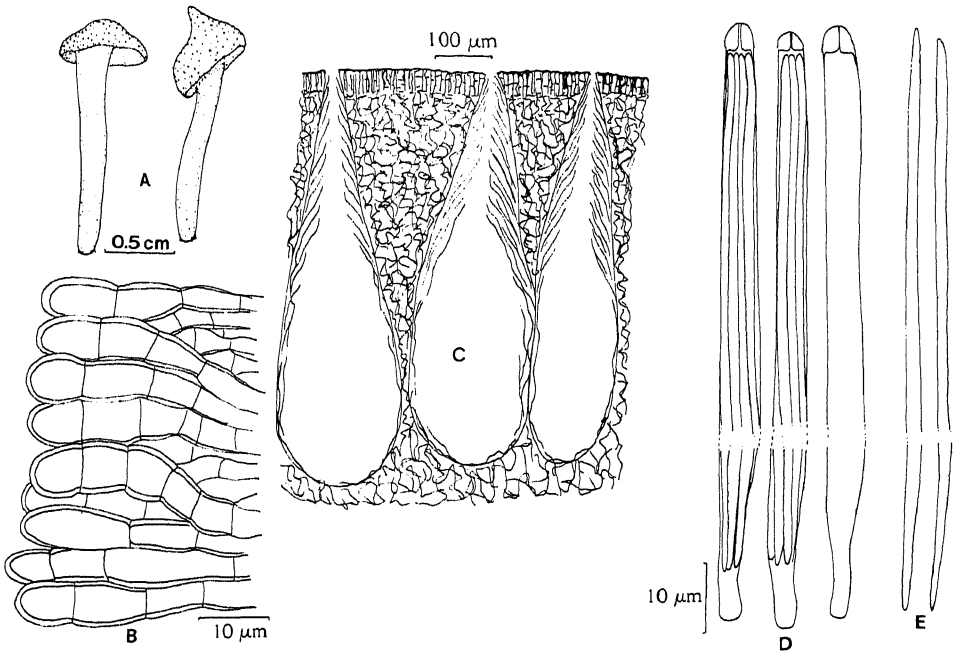


Figure 5. *Cordyceps cantharelloides*. Camera lucida drawings. **A.** Clavae. **B.** Outer cell wall of fertile head. **C.** Perithecia. **D.** Asci. **E.** Ascospores.

the cap of a *Cantharellus*, 10–11 × 3–5 mm. Outer wall of the fertile head, composed of a palisade of cylindrical thick-walled cells (figure 4B). Perithecia completely embedded, densely crowded papillae, protruding from the dorsal surface; narrowly obclavate, 260–380 × 110–140 μm, with a long neck, lined with paraphyse. Asci cylindrical, almost as long as the perithecial chamber, 140–160 × 4–5 μm, with thickened apical region or cap, swollen in the centre, narrowing towards the long tail region. Ascospores hyaline, filiform, multiseptate, guttulate, with pointed apices, not breaking into part-spores, 75–85 × 2–2·5 μm.

Anamorph not present, but fruitbodies parasitized by synnemata of the *Stilbella* type (figure 6). Synnemata consisting of smooth or hirsute stipe, sometimes terminating in a clavate slimy head. Phialides arising singly or in whorls of 2 to 3, either laterally from the hyphae of the synnema or terminally from the apex, flask-shaped with a long neck, 10–15 × 1·5–2 μm. Conidia usually developing in a viscous mucus, 1–4 per phialide, ellipsoidal to obovate, smooth-walled, hyaline, 1·5–3 × 1–1·5 μm.

Holotype on larva of Coleoptera in tree stem, Palora, Pastaza, Ecuador, 30 Nov. 1975, H C Evans (IMI 290521). Isotype consisting of one clava parasitised by *Stilbella* sp., in Herb. CBS, RS 1175.

The collecting area was a remote forest in the Andean foothills (*ca.* 1000 m alt.) in the Amazonian (eastern region of Ecuador). This species produces fruitbodies strikingly similar to the basidiocarps of some agarics, particularly to those of the genus *Cantharellus*. Indeed, during the initial collection, the two clavae which were emerging from a hole in a living tree (*ca.* 2 m above ground level) were mistaken for agarics and removed. Only then was it realised that they were growing out of the tunnel of a boring

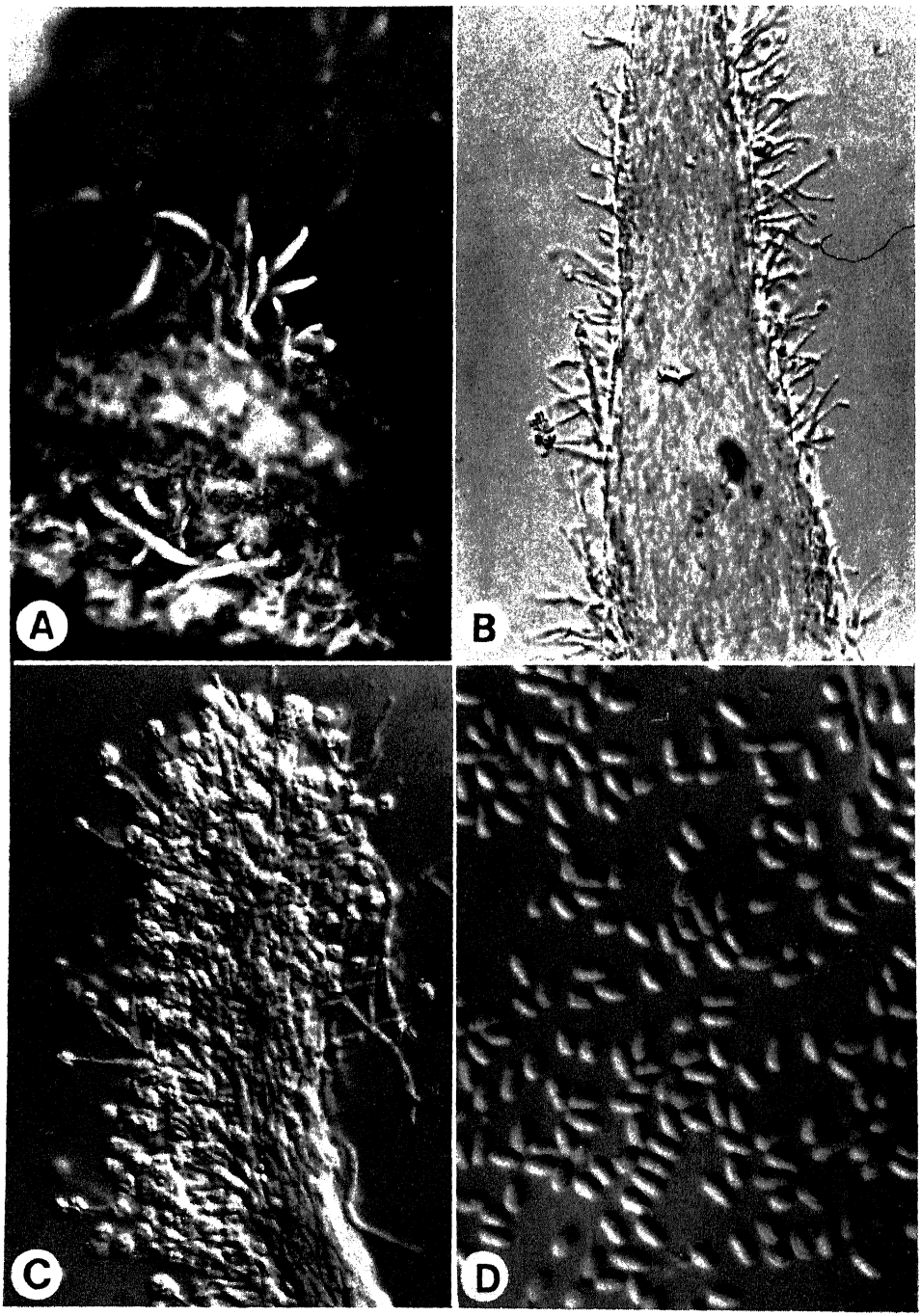


Figure 6. *Stilbella* species. **A.** Synnemata (× 20). **B–C.** Phialides along the synnema, (**B.** × 550, C. × 720) **D.** conidia (× 1800).

insect and that the clavae penetrated laterally and vertically into the tree bole (some 10–15 cm) and were attached to a large (7 × 0·8 cm) coleopteran larva. Due to the extreme hardness of the wood, the entire specimen took several hours to extract, which testifies to the boring ability of the insect (see also figure 3A). The characteristic shape of the clavae distinguishes this species from all known *Cordyceps* species (Kobayasi and Shimizu 1983).

It is also possible that this unusual fungus might be macroscopically mistaken for a *Hypomyces* species parasitic on an agaric, since the crowded, completely immersed perithecia with bluntly rounded, slightly protruding papillae are also typical of representatives of this genus.

The stromata were hyperparasitized by a *Stilbella*-like fungus. Petch (1924) and Kobayasi (1941) described *Stilbella* species (*S. barnesii* and *S. falcata*) and considered them as anamorphs of *Cordyceps* spp. without any firm evidence of the relationship. Following our examination of material in Herb K and an analysis of the original descriptions, we conclude that the *Stilbella* is hyperparasitizing the clavae of *C. barnesii* and *C. falcata* and that the fungus found on *C. cantharelloides* has a similar parasitic habit.

In his revision of *Stilbella* and related synnematous hyphomycetes, Seifert (in preparation) has shown that the species described here should be assigned a new specific epithet, since *Stilbella barnesii* and *S. falcata* are invalid names, being published without a Latin diagnose. Together with *Stilbella kervillei* (Samson *et al* 1984), these species should probably be excluded from *Stilbella* on the basis of phialide shape and synnema characters and placed in another genus possibly *Polycephalomyces* Kobayasi.

Acknowledgements

The authors thank Dr W Gams for helpful suggestions and the Latin descriptions. Mr Keith A Seifert kindly provided data on *Stilbella*.

References

Evans H C and Samson R A 1982 *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems. I. The *Cephalotes* (Myrmicinae) complex; *Trans. Br. Mycol. Soc.* **79** 431–453

Evans H C and Samson R A 1984 *Cordyceps* species and their anamorphs pathogenic on ants (Formicidae) in tropical forest ecosystems. II. The *Camponotus* (Formicinae) complex; *Trans. Br. Mycol. Soc.* **82** 127–150

Kobayasi Y 1941 The genus Cordyceps and its allies; *Sci. Rep. Tokyo Bunrika Daigaku* **B84** 53-260

Kobayasi Y and Shimizu D 1983 *Iconography of vegetable wasps and plantworms* (Osaka: Hoikusha Publishing Co.)

Petch T 1924 Studies in entomogenous fungi. IV. Some Ceylon *Cordyceps*; *Trans. Br. Mycol. Soc.* **10** 28–45

Samson R A, Rombach M C and Seifert K A 1984 *Hirsutella guignardii* and *Stilbella kervillei*, two troglobiotic entomogenous hyphomycetes; *Persoonia* **12** 123–134

Speare A T 1920 On certain entomogenous fungi; *Mycologia* **12** 62–76

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 319–339.
© Printed in India.

Problems and prospects in the systematics of the Ascomycotina

D L HAWKSWORTH
Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF, UK

Abstract. The classification of the Ascomycotina remains one of the major problems in systematic mycology. A survey of the various systems proposed over the last five decades is presented identifying their key aspects. A table including outlines of 20 systems is provided. Constraints to developing a generally acceptable system are identified as arising from the size of the group, its geological age, assumption of phylogenetic concepts, data availability, convergence, delimitation of genera, and taxa remaining to be discovered. Progress towards an acceptable system must pay due regard to the users of taxonomy and have strong elements of stability. Data collection could be approached by concerted efforts of groups of specialists tackling particular orders in turn. It seems most appropriate to progress by building up the hierarchy from maximum information content groups, concentrating on delimiting monophyletic units in the ranks of order and family, and restricting hypothetical overall systems to specialist debate rather than recommending them for general use.

Keywords. Fungi; Ascomycotina; lichens; systematics; classification.

1. Introduction

The last three decades have seen the systematics of the Basidiomycotina and Deuteromycotina placed on an increasingly secure base, with substantial areas of consensus as to higher categories meriting recognition and generic limits. In contrast, the casual observer might with some justification have the impression that the situation in the Ascomycotina has become increasingly unstable. Symptomatic is the title of the meeting "Ascomycetes in Disarray" organized by the British Mycological Society on 17 November 1984. Luttrell's (1951) remark that "The classification of the Ascomycetes constitutes perhaps the most difficult taxonomic problem in mycology" is unfortunately still valid over 30 years after it was made.

The object of this contribution is to both examine how the present situation has arisen, identify the constraints, and establish guidelines as to how we might proceed towards a generally acceptable system.

2. Proposed systems

A selection of systems for the Ascomycotina used during the last five decades is compiled in table 1, from which the extent of variation even amongst current workers will be clear. Table 1 only includes ranks down to order; had space permitted the incorporation of family names, the discrepancies would be even more marked.

Clements and Shear (1931) aimed at a practical system with ten orders and no higher categories within the group. Their concepts of some orders were, however, extremely broad. For example, their Sphaeriales even included the Cyttariaceae, and their Pezizales the Caliciaceae, Helotiaceae, Parmeliaceae and Patellariaceae. The main characters these authors used to separate orders were the appearance of the ascus-containing structure (*ascoma*), particularly whether it was stromatic, more or less

Table 1. Classifications proposed for the Ascomycotina 1931–84.

Clements and Shear (1931)	Nannfeldt (1932)	Miller (1949)	Luttrell (1951)	von Arx & Müller (1954, 1962)	Chadefaud (1960)
Laboulbeniales	DIPLOBIONTICAE	HEMIASCOMYCETES	SYNASCOMYCETES	Endomycetales	LABOULBENIOMYCETES
Gymnascales	HAPLOBIONTICAE	Endomycetales	Protomycetales	Taphrinales	Laboulbeniales
Perisporiales	Endomycetales	Exoascales			DISCOMYCETES
Sphaeriales*	HEMIASCI	EUASCOMYCETES	HEMIASCOMYCETES	UNITUNICATAE	*Lécanoriens*
Dothideales*	EUASCOMYCETES	*Plectomycetes*	Dipodascales	Plectascales	Lecanorales*
Microthyriales	Plectascales	Eurotiales	Endomycetales	Erysiphales	*Hélotiens*
Phacidiales*	*Ascoloculares*	Erysiphales	Taphrinales	Clavicipitales	Hypodermales
Pezizales*	Myriangiales	Myriangiales		Sphaeriales	Helotiales
Agyriales	Pseudosphaeriales	*Pyrenomycetes*	EUASCOMYCETES	Phacidiales	*Léotiens*
Tuberales	Hemisphaeriales	Pseudosphaeriales	**Bitunicatae**	Helotiales	Tympanidales
	Trichothyriales	Dothideales	Myriangiales	Ostropales	Ostropales
	Ascohymeniales	Microthyriales	Dothideales	Pezizales	Leotiales
	Pezizales	Hysteriales	Pseudosphaeriales	Tuberales	Sarcoscyphales
	Ostropales	Hypocreales	Hysteriales	Laboulbeniales	Caliciales*
	Helotiales	Lophiostomatales	Trichothyriales		*Pezizéens*
	Lecanorales*	Sphaeriales	**Unitunicatae**	BITUNICATAE	Pezizales
	Sphaeriales	Laboulbeniales	*Plectomycetes*	Myriangiales	"aberrant"
	Diaporthales	*Discomycetes*	Aspergillales	Pseudosphaeriales	Tuberales
	Valsales	Ostropales	Microascales	Hysteriales	Elaphomycetales
	Coronophorales	Helotiales	Onygenales	Dothiorales	PYRENOMYCETES
	Clavicipitales	Pezizales	*Pyrenomycetes*		*Dothidéens*
	Incertae sedis	Tuberales	Xylariales		Pleosporales
	Exoascales		Hypocreales		Dothiorales
	Laboulbeniales		Diaporthales		Myriangiales
			Erysiphales		*Coronophoriens*
			Coronophorales		Coronophorales
			Coryneliales		Nectriales
			Laboulbeniomycetes		*Sphaeriacéens*
			Laboulbeniales		Diaporthales
			Discomycetes		Sordariales
			Pezizales		Nectriales
			Tuberales		Diatrypales
			Cyttariales		Xylariales
			Ostropales		Hyponectriales
			Helotiales		Glomerellales
			Lecanorales*		*Clavicipitiens*
			Caliciales*		Clavicipitales
					"aberrant"
					Erysiphales
					Eurotiales
					HÉMI-ASCOMYCETES
					Endomycetales
					Dipodascales

* = Orders including at least some lichen-forming fungi as circumscribed by the authors indicated.

bsent, ostiolate, disc-like, scale-like or elongate. Although such features are valuable n diagnostic keys, more critical studies of their development and structure were clearly equired. Clements and Shear (1931) recognized that knowledge at that time was grossly inadequate, but stressed one point which should not be overlooked by present vorkers, that "to follow a conservative course in the recognition and limitations of rders and families seems to . . . best serve the . . . interests of users" and that a epeatedly changing system "constantly shifts the foundations of mycology to the isadvantage of practically everyone".

A major attempt to place ascomycete systematics on a firmer base was that of Nannfeldt (1932), who while not developing a complete system, endeavoured to distinguish two major groups on the basis of the ontogeny of the ascoma; the Ascoloculares in which the asci developed in cavities in a pre-formed stroma, and the

Gäumann (1964)	Hale (1967)	Kreisel (1969)	Ainsworth *et al* (1971)	Henssen & Jahns (1973)
PROTOTUNICATAE	ASCOMYCETIDAE	ENDOMYCETES	HEMIASCOMYCETES	ASCOHYMENIALE PILZE
Endomycetales	Lecanorales*	Endomycetales	Endomycetales	Caliciales*
Protomycetales	Sphaeriales*	Taphrinales	Protomycetales	Lecanorales*
Ascosphaerales	Caliciales*	ASCOMYCETES	Taphrinales	Lecanorineae*
Aspergillales		*Euascomycetidae*		Lichinineae*
Microascales		Ascosphaerales	PLECTOMYCETES	Peltigerineae*
Onygenales	LOCULOASCOMYCETIDAE	Eurotiales	Ascosphaerales	Teloschistineae*
Meliolales	Myriangiales*	Gymnascales	Eurotiales	Physciineae*
Laboulbeniales	Pleosporales*	Tuberales	Microascales	Pertusariineae*
	Hysteriales*	Pezizales	Erysiphales	Gyalectales*
EUTUNICATAE		Helotiales	Meliolales	Ostropales*
Unitunicatae		Microascales		Ostropineae*
Erysiphales	Included only	Laboulbeniales	PYRENOMYCETES	Graphidineae*
Clavicipitales	orders with lichen-forming fungi	Xylariales	Hypocreales	Sphaeriales*
Sphaeriales		Erysiphales	Clavicipitales	Verrucariales*
Diaporthales		Hypocreales	Sphaeriales	
Xylariales		Sphaeriales	Coryneliales	ZWISCHENGRUPPE
Coronophorales		*Loculoascomycetidae*	Coronophorales	Arthoniales*
Phacidiales		Myriangiales		
Helotiales		Capnodiales	DISCOMYCETES	ASCOLOCULARE PILZE
Pezizales		Dothideales*	Phacidiales	Pleosporales*
Tuberales		Coronophorales	Helotiales	Dothideales*
Taphrinales		Phacidiales	Ostropales	
Bitunicatae		Lecanorales*	Pezizales	Included only orders with lichen-forming fungi.
Myriangiales		Hysteriales*	Tuberales	
Pseudosphaeriales		Caliciales*		
Hysteriales			LABOULBENIOMYCETES	
Dothiorales			Laboulbeniales	
			LOCULOASCOMYCETES	
			Myriangiales	
			Pleosporales	
			Hysteriales	
			Dothideales	
			Capnodiales	
			Microthyriales	

Table 1 (*contd.*)

Ascohymeniales where the asci develop as a hymenium and not in a pre-formed stroma. Nannfeldt's concepts were quickly followed by some authors (*e.g.* Bessey 1935), but did not then receive any general acceptance. At this time highly pertinent observations on the ontogeny of pyrenomycetous fungi were being made by Chesters (1938), the full significance of which only started to be appreciated in the 1970's.

Miller (1949) recognized that "important fundamental characters such as those of the ascus, or those of the ascocarp [ascoma] centrum, have been largely ignored in establishing relationships" and that "this has resulted in artificial groupings and keys of little practical use". In this paper he developed his earlier ideas and endeavoured to construct a system "based primarily on characters of the ascus correlated with ascocarp [ascoma] centrum characters". However, in contrast to Nannfeldt (1932), his major groups were based on rather superficial features; whether a specialized ascoma

Table 1 (*continued*)

Ainsworth *et al.* (1973)	Wehmeyer (1975)	Barr (1976)	von Arx (1979)	Eriksson (1982)
HEMIASCOMYCETES	PROTOASCOMYCETES	HEMIASCOMYCETES	Gymnoascales	EUASCOMYCETIDAE
Endomycetales	Endomycetales	Protomycetales	Eurotiales	Group A
Protomycetales	Hemiascales	Taphrinales	Sphaeriales	Lecanorales*
Taphrinales	Taphrinales	Spermophthorales	Phacidiales	Gyalectales*
		Cephaloascales	Helotiales	Pertusariales*
PLECTOMYCETES	CARPOASCOMYCETES	Dipodascales	Pezizales	Peltigerales*
Eurotiales	*Loculoascomycetes*	Ascoideales	Erysiphales	
	Microthyriales		Dothideales	
PYRENOMYCETES	Hysteriales	EUASCOMYCETES		Group B
Erysiphales	Myriangiales	*Plectoascomycetidae*		Ostropales
Meliolales	Dothideales	Eurotiales		Graphidales*
Coronophorales	Pleosporales	*Laboulbeniomycetidae*		Pezizales
Sphaeriales	*Euascomycetes*	Spathulosporales		Elaphomycetales
	Plectascales	Laboulbeniales		Cyttariales
DISCOMYCETES	Melanosporales	*Parenchemycetidae*	The Endomycetales	Helotiales*
Mediolariales	Xylariales	Erysiphales	and Taphrinales	Medeolariales
Cyttariales	Allantosphaeriales	Meliolales	excluded from the	Phacidiales
Tuberales	Diaporthales	Diaporthales	Ascomycetes. Von	Diaporthales
Pezizales	Hypocreales	Sordariales	Arx (1981) placed	Sordariales
Phacidiales	Laboulbeniales	Coronophorales	the Endomycetales	Diatrypales
Ostropales	Clavicipitales	*Anoteromycetidae*	as Endomycetes	Xylariales
Helotiales	Erysiphales	Ostropales*	and included the	Polystigmatales
	Sphaeriales	Gyalectales*	Taphrinales in	Hypocreales
LABOULBENIOMYCETES		Clavicipitales	the Ustomycetes.	Hypomycetales
Laboulbeniales		Hypocreales		Clavicipitales
		Chaetomiales		
LOCULOASCOMYCETES		*Elaphomycetidae*		Group C
Myriangiales		Mediolariales		Lecanidiales*
Dothideales		Cyttariales		Arthoniales*
Pleosporales		Coryneliales		Opegraphales*
Hysteriales*		Pezizales		Verrucariales*
Hemisphaeriales		Tuberales		Pyrenulales*
		Helotiales*		Dothideales*
		Lecanorales*		
		Caliciales*		Group D
		Rhytismatales		Coryneliales
		Phyllachorales*		Caliciales*
		Xylariales		Ascosphaerales
				Onygenales
		LOCULOASCOMYCETES		Eurotiales
		Loculoplectascomycetidae		Microascales
		Myriangiales*		Ophiostomatales
		Loculoparenchemycetidae		Taphrinales
		Asterinales		Protomycetales
		Dothideales*		Endomycetales
		Loculoanoteromycetidae		
		Chaetothyriales		LABOULBENIOMYCETIDAE
		Verrucariales*		Laboulbeniales
		Loculoedaphomycetidae		Spathulosporales
		Hysteriales*		
		Pleosporales*		37 accepted families
		Melanommatales*		were not assigned to
				any of the above orders.

was absent (Hemiascomycetes), closed (Plectomycetes), opening by a pore or slit (Pyrenomycetes) or open (Discomycetes). The ontogenetic features stressed by Nannfeldt were used in the separation of orders and not the higher taxa. Hansford (1946) was less convinced of the importance of the centrum structure and regarded

Hawksworth *et al.* (1983)	Barr (1983)	Eriksson (1983)	Locquin (1984)	
Arthoniales*	*ENDOMYCOTA*	Lecanorales*	SPERMOPHTHOROMYCETES	MAZAEDIOMYCETES
Ascosphaerales	ENDOMYCETES	Teloschistales*	Spermophthorales	*Plectomazaediomycetidae*
Caliciales*	Endomycetales	Gyalectales*	ENDOMYCETES	Warcupiellales
Clavicipitales	Protomycetales	Pertusariales*	Endomycetales	*Lipsanomazaediomycetidae*
Coryneliales	*TAPHRINOMYCOTA*	Peltigerales*	Dipodascales	Gymnoascales
Cyttariales	TAPHRINOMYCETES	Ostropales	Cephaloascales	*Epitheliomazaediomycetidae*
Diaporthales	Taphrinales	Graphidales*	HEMIASCOMYCETES	Chaetoiniales
Diatrypales	*ASCOMYCOTA*	Caliciales*	Taphrinales	Microascales
Dothideales*	ASCOSPHAEROMYCETES	Pezizales	SYNASCOMYCETES	Rhytidosporales
Elaphomycetales	Ascosphaerales	Elaphomycetales	Protomycetales	Zopfiellales
Endomycetales	LABOULBENIOMYCETES	Cyttariales	Pericystales	Eurotiales
Erysiphales	Laboulbeniales	Helotiales*	ASCOBLASTOMYCETES	Ophiostomatales
Eurotiales	ASCOMYCETES	Medeolariales	Saccharomycetales	Thermoascales
Graphidales*	*Parenchymatomycetidae*	Rhytismatales	LABOULBENIOMYCETES	Penicillopsiales
Gyalectales*	Eurotiales	Diaporthales	*Laboulbeniomycetidae*	Meliolales
Gymnoascales	Elaphomycetales	Sordariales	Laboulbeniales	Mycorhynchales
Helotiales*	Spathulosporales	Microascales	Peritschiellales	Elaphomycetales
Hypocreales	Coryneliales	Ophiostomatales	Coreomycetales	Caliciales*
Laboulbeniales	Erysiphales	Diatrypales	Zodiomycetales	NASSASCOMYCETES
Lecanidiales*	Diaporthales	Xylariales	*Spathulosporomycetidae*	*Loculoplectoascomycetidae*
Lecanorales*	Corynophorales	Amphisphaeriale	Spathulosporales	Myriangiales
Microascales	Microascales	Halosphaeriales	ANELLASCOMYCETES	*Loculoparenchyemycetidae*
Opegraphales*	Calosphaeriales	Calosphaeriales	*Discomycetidae*	Capnodiales
Ophiostomatales	Sordariales	Trichosphaeriales	Pezizales	Dothideales
Ostropales*	*Edaphomycetidae*	Phyllachorales	Tuberales	*Loculoanoteramycetidae*
Peltigerales*	Onygenales	Hypocreales	Medeolariales	Trichothyriales
Pertusariales*	Medeolariales	Eurotiales	Cyttariales	Verrucariales*
Pezizales	Pezizales	Hypomycetales	Phacidiales	Lecanorales*
Polystigmatales	Cyttariales	Clavicipitales	Ostropales	*Loculoedaphomycetidae*
Pyrenulales*	Hypodermatales	Patellariales*	Tympanidales	Perisporiales*
Rhytismatales	Phacidiales	Arthoniales*	Thelotrematales*	Lophiostomatales*
Sordariales	Caliciales*	Opegraphales*	Hypodermatales	Hysteriales*
Spathulosporales	Helotiales*	Verrucariales*	Leotiales	
	Ostropales*	Pyrenulales*	Pezizellales	
Sphaeriales*	Lecanorales*	Dothideales*	*Pyrenomycetidae*	
Taphrinales	Meliolales	Erysiphales	Erysiphales	
Teloschistales*	Clavicipitales	Coryneliales	Coryneliales	
Verrucariales*	Hypocreales	Ascosphaerales	Hypomycetales	
	Xylariales	Onygenales	Nectriales	
	Phyllachorales	Taphrinales	Hypocreales	
	Trichosphaeriales	Protomycetales	Hyponectriales	
	LOCULOASCOMYCETES	Endomycetales	Polystigmatales	
	Loculoparenchymatomycetidae	Laboulbeniales	Diatrypales	
	Myriangiales*	Spathulosporales	Diaporthales	
	Asterinales		Sordariales	
	Dothideales*		Chaetosphaeriales	
	Loculoedaphomycetidae		Xylariales	
	Pleosporales		Ceratostomellales	
	Melanommatales		Glomerellales	
	Verrucariales*		Coronophorales	
	Chaetothyriales		Sphaeriales	

"the origin of the mucus within the locules . . . of little importance from the systematic point of view".

Nannfeldt (1932) re-iterated the views of several earlier workers (*e.g.* Clements 1909) that the lichen-forming fungi were a biological and not a taxonomic group and so

should be integrated into the system for other ascomycetes, but this view was not shared by most lichenologists of that time (*e.g.* Zahlbruckner 1926; Watson 1929) even though in 1856 Berkeley had been convinced of the close affinities and difficulty of drawing any clear dividing line (Lindsay 1869). The first author to seriously endeavour to integrate some of the lichen-forming families according to Nannfeldt's concepts was Santesson (1949, 1952, 1953).

Müller and von Arx (1950) studied the Ascoloculares further, provisionally accepting the orders Myriangiales, Pseudosphaeriales, Dothiorales, Asterinales, and surprisingly the Erysiphales and Tuberales. Simultaneously and quite independently Luttrell (1951) was not only taking note of lichenized groups but developing the concepts of Nannfeldt and Miller. On the basis of published evidence he concluded that the Ascoloculares and Ascohymeniales developmental types of Nannfeldt (1932) could be correlated with the presence of "bitunicate" and "unitunicate" asci respectively. He placed primary emphasis on this character and introduced the new terms Bitunicatae and Unitunicatae for what were substantively the same groups as Nannfeldt, although he did refer the plectomycete families to the Unitunicatae rather than excluding them from that dichotomy. In addition, Luttrell provided a most skilful synthesis of the published information available on centrum and ascus structures, especially for the pyrenomycete groups. His ideas were quickly adopted and utilized by his contemporaries, including, although in a somewhat simplified scheme, the classic works of Müller and von Arx (von Arx and Müller 1954; Müller and von Arx 1962); these latter authors excluded the Endomycetales and Taphrinales from the main groups of ascomycetes, something they were to increasingly stress in future years. Luttrell (1955) subsequently reviewed the nomenclature of the higher taxa to be recognized and introduced the subclass name Loculoascomycetes for the Ascoloculares (syn. Bitunicatae), re-ascerting that bitunicate species appeared to represent a monophyletic line distinct from the Euascomycetes. His confidence in this had been increased as several previously reported cases of a lack of correlation between ascolocular ascomata and bitunicate asci had proved to be erroneous. Phylogenetic diagrams were also now included, showing some linkages that later data suggests are most improbable.

About this time some authors felt that the fungal partners in lichen associations should be classified in the fungal system and given quite separate names. This is not permitted under the Code and need not concern us here. This debate has been reviewed elsewhere (Hawksworth 1978).

Luttrell's system did not attain immediate acceptance by all workers. Chadefaud (1960), who with his co-workers has made an unparalleled contribution to our knowledge of ascus structure, retained a system more akin to that of Miller (1949) but did stress the special position of some groups (*e.g.* Clavicipitales, Lecanorales) and acknowledged that some orders did not rest easily in his groupings. Gäumann (1964) recognized two subclasses, the Prototunicatae with a "primitive" ascus structure and passive release of the ascospores and the Eutunicatae with functionally differentiated ascus structures and active ascospore discharge. The Eutunicatae were then divided into two series largely following Luttrell's ideas, the Unitunicatae and Bitunicatae. However, the placement of some orders resulted in the juxtaposition of fungi that would now be regarded as remote, for example the Onygenales and Meliolales, and the Tuberales and Taphrinales.

Hale (1967) endeavoured to refer the lichenized orders to the two main groups

distinguished by Nannfeldt and Luttrell, using the subclass names Ascomycetidae and Loculoascomycetidae, but did not discuss placements in detail. This period did, however, see a marked increase in the studies being made of the ontogeny of ascomata and ascus structure. The wealth of data that started to become available on both lichen-forming (*e.g.* Chadefaud *et al* 1970; Henssen 1963; Janex 1971; Letrouit-Galinou 1967, 1969; Richardson 1970; Vězda 1968) and on non-lichenized groups (*e.g.* Bellemère 1968, 1971; Parguey-Leduc 1966) made it clear that a satisfactory integration of the lichen-forming groups into the system developed for other ascomycetes could not be easily accomplished. Too many developmental types and variations in ascus structure were starting to emerge.

Kreisel (1969) produced a thought-provoking system from two points of view: recognition of a group Endomycetes (which was to be taken up later) and the placement of the Caliciales, Coronophorales, Lecanorales and Phacidiales in the Loculoascomycetidae. In recognizing some of these affinities he was considerably in advance of most of his contemporaries.

The sixth edition of the *Dictionary of the Fungi* (Ainsworth *et al* 1971) adopted a more conservative classification, closest to that of Miller (1949) but accepting the Laboulbeniomycetes and Loculoascomycetes as distinct subclasses. The refaral of lichenized groups to this system was deliberately avoided in this edition (although lichen-forming genera were now listed for the first time) because of the uncertainties as to how this could be accomplished in view of apparent anomalies and insufficient data on many genera. The detailed account of ascomycete orders and families with keys to the genera included in *The Fungi: an advanced treatise* (Ainsworth *et al* 1973) was arranged by the subclasses utilized in the 1971 *Dictionary*, although a few contributors (*e.g.* Luttrell) did include some lichenized genera. Important reviews of ascus types were prepared at this time by Chadefaud (1973) and Letrouit-Galinou (1973) which again stressed the diversity to be found, highlighting the almost tritunicate archaeascus type seen in the Lecanorales and interpreting it as having a more primitive nature than the unitunicate and bitunicate types generally distinguished.

In that same year a most remarkable work appeared, that of Henssen and Jahns (1973) on the lichen-forming fungi. This survey not only synthesized a rapidly growing body of knowledge but included a wealth of new ontogenetic observations. These authors found that their observations could not be entirely reconciled with previous systems. Complex ascus types, some with a bitunicate structure and discharge occurred in ascohymenial developmental types (*e.g.* Peltigerineae) and families that seemed close to some Sphaeriales (*e.g.* Strigulaceae); there was a substantial group with intermediate ascomatal types ("Zwischentyp") and bitunicate asci (the Arthoniales, treated there in a broad sense to include Lecanactidaceae and Opegraphaceae). Poelt (1974), however, chose to recognize eight orders amongst the lichen-forming ascomycetes but did not refer them to higher categories; nine families he accepted could not be definitely referred to any of those orders, something indicative of the problems starting to appear.

von Arx and Müller (1975) provided most useful keys to the genera with bitunicate asci, referring these to a single order, Dothideales, rather than to the 14 proposed at that time. They found a subdivision of the bitunicate ascomycetes based on taxonomically reliable characters such as the ascus structure and disposition of the asci within ascomata to be difficult to apply. Thirty-four families were accepted, dispersed between two new suborders, the Dothideineae (ascomata opening by dehiscence or

rupture; asci clavate or nearly spherical) and Pseudosphaeriineae (ascomata usually ostiolate; asci usually cylindrical or saccate). Such a broad view of that order was not shared by Wehmeyer (1975) who in a posthumous work accepted five orders within his Loculoascomycetes; he also introduced a new divisional name Carpoascomycetes to accommodate all groups where the asci or hymenium is enclosed within a sterile tissue of vegetative hyphae (*i.e.* all except the Protoascomycetes).

New concepts were introduced by Barr (1976), who paid particular attention to the incorporation of the lichen-forming groups on the basis of the information presented by Henssen and Jahns (1973). She not only recognized three classes, Hemiascomycetes, Euascomycetes and Loculoascomycetes but proceeded to divide the latter two subclasses into a series of subclasses based primarily on the centrum types. The Euascomycetes included the Plectoascomycetidae (asci small, deliquescing, irregularly arranged, mainly in cleistothecia), Laboulbeniomycetidae (asci thin, deliquescing, one or few in minute perithecia), Parenchemycetidae (centrum pseudoparenchymatous, no true paraphyses), Anoteromycetidae (centrum with lateral or apical paraphyses, basal paraphyses only in apothecial forms), and Elaphomycetidae (centrum with hymenial paraphyses), while the Loculoascomycetes comprised the Loculoplectascomycetidae (asci in uniascal locules), Loculoparenchemycetidae (interascal tissue thin-walled, of locule cells, or absent), Loculoanoteromycetidae (pseudoparaphyses absent, with apical and lateral paraphyses, *i.e.* periphysoids), and Loculoedaphomycetidae (interascal tissue pseudoparaphyses of some type). Thirty-eight orders were accepted, more than by any previous worker. Barr (1979) provided a more detailed account of the Loculoascomycetes according to her scheme with keys to the families and lists of representative genera.

Such a large number of orders was unacceptable to von Arx (1979) who had become very concerned at the emphasis on the presence or absence of interascal tissues. More importantly his studies had shown that intermediates existed between the Euascomycetes and Loculoascomycetes. For example, he produced evidence from both ascus structure and nature of the anamorphs indicating close affinities between some genera normally referred to families placed in different subclasses (*e.g. Diaporthe, Discostroma, Massaria, Massariovalsa* and *Melanconis*). von Arx concluded that the Eu and Loculoascomycetes separation was superfluous and that the ascomycetes should be arranged in a limited number of orders without higher categories; only eight orders were accepted (lichen-forming families were omitted). He also pointed out that this idea was not new; Franz Petrak (1886–1973) who had seen perhaps a wider range of ascomycetes from different regions than most of his contemporaries, never accepted the bitunicate/unitunicate division because he was convinced intermediates occurred.

In a later work, von Arx (1981) excluded from the Ascomycetes (i) the Endomycetales, treating them as a separate class, the Endomycetes, and also (ii) the Taphrinales, which he referred to as the Ustomycetes. Subsequent ultrastructural studies of the cell walls, however, showed that *Protomyces* and *Taphrina* had ascomycete-like cell walls with a distinct electron-transparent layer leading to their inclusion with the Endomycetes rather than the Ustomycetes which have electron dense layers in their walls (von Arx *et al* 1982). Ultrastructure was consequently recognized as having a key role in determining what fungi should be included within the Ascomycotina.

Additional TEM work was now starting to show the structural basis to the observations on asci of lichenized groups made by Chadefaud and his colleagues

(Honegger 1978a,b,c, 1980, 1982a,b, 1983) and it was becoming increasingly clear that ascus types varied extensively. A useful diagram of the types then becoming separable is provided in Sherwood (1981) and a most important series of papers on ascus and centrum types was edited by Reynolds (1981). The key fact which emerged from this latter survey was that while the basic concepts of ascus and centrum types were the key to ascomycete systematics, the variation in these between groups was much more than generally appreciated into the early 1970's. However, it has also become clear that great care is needed in ascus structure studies as some of the described complexities have been based on degenerate asci from old material (Honegger 1978b); fresh collections are essential for sound work in this field.

As an appendix to an extremely important study of the type species of genera which were types of families reported to have bitunicate asci, Eriksson (1981) arranged the ascomycete family names in a series of 109 clades which he considered monophyletic; 30 were excluded as of uncertain position. Eriksson stressed that the first step in the production of a system for the ascomycetes must . . . "be the recognition of natural families of extant ascomycetes and the grouping of these families into monophyletic entities", *i.e.* a system should be rebuilt downwards from this level rather than upwards on the basis of assumed phylogenies. This system was formalized by Eriksson (1982) who distinguished two subclasses, the Euascomycetidae and the Laboulbeniomycetidae. Within the former four groups of orders were recognized based on the type of ascus: Group A archaeasceous, Group B unitunicate, Group C bitunicate (excluding bilabiate and semi-fissitunicate types, referred to A), and Group D pseudoprototunicate. In this work 228 families were accepted, dispersed through 39 orders.

Chadefaud (1982a) stressed the importance of two concepts in discussing ascomatal organization: the carpocentre, first introduced by Chadefaud (1960), for the tissue within which the ascogonia arise, and the gynocarp, newly proposed for the carpocentre and its enveloping tissues. These concepts were applied to a wide range of ascomycetes (lichenized and non-lichenized) producing apothecia (discocarps) by Chadefaud (1982b). He concluded that the group was highly polyphyletic with at least five ontogenetic lines, which could perhaps be reduced to two if ascal features were stressed, centred on (i) a hypodermian line (asci with an ovigal apex but no apical apparatus) and (ii) an operculate pezizean line (asci with an apical plug or ring and possibility of an apical canal). However, these concepts were not developed here into a new system for the apotheciate ascomycetes.

In view of the differences in systems that were becoming apparent, for the seventh edition of the *Dictionary of the Fungi* (Hawksworth *et al* 1983) it was felt most appropriate to follow von Arx (1979) in not accepting ranks above that of order. Thirty-seven orders were treated, however, 16 of which included or consisted entirely of lichenized representatives. To have effectively selected one of the alternative hierarchical systems for general use would have been insidious and could mislead, implying that more was known of phylogenetic relationships than could really be supported by the evidence. This view was followed by Eriksson (1983) who formally withdrew the subclass names of his previous system and "Groups" but now accepted 44 orders.

Meanwhile Barr (1983) revised her previous scheme. She followed von Arx in placing the Endomycetales and Taphrinales in isolated positions in a rank comparable to Ascomycota, as Endomycota and Taphrinomycota respectively (see von Arx *et al*

1982 cited above). Within the re-defined Ascomycota, the Ascosphaerales and Laboulbeniales were now treated as independent subclasses, and the number of subgroups within both the Ascomycetes and Loculoascomycetes was reduced to two based on centrum types as evidenced in the nature of the interascal tissues; 38 orders were accepted and keys to these provided. Sivanesan (1984), however, adopted a more conservative view of the loculoascomycete groups; he recognized seven orders, as did Barr (1983), but he retained the Capnodiales and Hysteriales and did not accept the Melanommatales and Verrucariales.

To conclude this section, passing mention must be made of the unfortunate publication of Locquin (1984). Many of the higher categories he recognized were based on derived features (*e.g.* deliquescing asci characterizing the Mazaediomycetes), remote taxa are juxtaposed (*e.g.* Verrucariales and Lecanorales), and many families are referred to quite inappropriate orders (*e.g.* the Parmeliaceae to Thelotrematales). A detailed discussion of this work would not be pertinent here; in summary, with respect to the ascomycetes, the work is best passed over by all but those that are concerned with cataloguing newly introduced names.

3. Constraints to developing an acceptable system

The variations in the systems proposed for the Ascomycotina, even within recent decades, are substantial as will be evident from the preceding section and table 1. If a generally acceptable system is to be developed, it is first appropriate to address the question why this is such a sysephian task in comparison with other groups of the Fungi.

3.1 *Numbers of Genera and Species*

Figures compiled in the *Dictionary of the Fungi* (Hawksworth *et al* 1983) suggest that there are 2720 genera (2810 synonyms) and 28650 species currently accepted in the Ascomycotina. These figures may be compared with the 1100 genera (910 synonyms) and 16000 species of Basidiomycotina, and 1680 genera (1270 synonyms) and 17000 species of Deuteromycotina. Even within the small area of the British Isles 1100 genera and 5100 species of ascomycetes are accepted (Cannon *et al* 1985). It is consequently not possible for a single mycologist to be fully conversant with the distinguishing characters of all but a relatively small number of genera at any one time. In the preparation of an overall scheme, authors consequently have to base a significant part of it not on their personal familiarity with the organisms but on the published results of others, a hazardous occupation. Most ascomycetologists also tend to limit their research activities to either restricted taxonomic groups (*e.g.* Hypocreales, Dothideales), biological groups (*e.g.* lichen-forming, marine, wood and bark inhabiting), or geographical regions (*e.g.* single countries, neotropics), and find it difficult to keep abreast of the current developments in knowledge over the group as a whole. The number of mycologists with a working knowledge of at least some lichenized fungi is particularly meagre, but a satisfactory grasp of the extent of the variation in this group is critical as it comprises almost half of all known Ascomycotina and includes centrum and ascus types not or scarcely known in non-lichenized groups.

.2 *Geological age*

The Ascomycotina may have been one of the first groups of fungi to evolve, and may well have its origins in the earliest geological periods. Certainly by Permo-Triassic times many existing genera, species or even chemical races appear to have evolved (Sheard 1977; Sherwood-Pike 1985; Tehler 1983), well before the rise of the flowering plants and mammals. In studying the extant ascomycetes, the possibility that some ancient lines may have been lost altogether, and that the present taxa may be so derived to have lost all but the slightest of ancestral traits must be borne in mind. There is a danger of searching for something which may have become extinct several hundred million years ago, and labelling as "ancestral" characters which cannot certainly be identified as such.

.3 *Phylogenetic concepts*

In the absence of an adequate fossil record (although there are indications that more can be gained from this than is generally assumed), concepts of phylogeny in the Ascomycotina must largely be deduced from representatives living today. There is a danger of hypotheses assuming a key position in the classification, with groups being divided according to what the hypotheses would predict; any groups not in accordance with this are either "squeezed" into inappropriate categories or treated as "aberrant" or "highly derived" and disregarded. For example, if Luttrell's (1951, 1955) concept of distinct unitunicate and bitunicate lines were correct, separation into two groups would be expected to be possible. However, if Cain's (1972) view that the main ascus types arose independently from algae were true, then a larger number of monophyletic groups corresponding to those types would be expected. In a similar manner, assuming that *Taphrina*, *Peltigera*, *Rhizocarpon* or *Spathulospora* is representative of an ancestral line may dictate the arrangement proposed. The result is a range of systems, incompatible to varying degrees, all of which are based on intuitive rather than substantiated hypotheses. The evidence to decide unequivocably which is "correct" may not be obtainable, so a range of possibilities can result, none of which can be ruled out as certainly "wrong".

.4 *Available data*

In view of the emphasis placed on centrum and ascus types in recent decades, it might have been assumed that substantial critical information was available on these characters in at least representatives of all major families within the Ascomycotina. Unfortunately this is far from the case, and even at the level of order gaps remain (table 2); for instance, data on ascus structure and function is inadequate in 13 of the 37 orders listed. As examples of additional orders and genera are studied critically new information is being obtained which shows that the previous interpretations were either too simplistic or erroneous. Recent examples include the demonstration of bissitunicate ascus discharge in *Rhizocarpon* (Honegger 1980), the combination of a loculoascomycete centrum and apparently unitunicate asci in *Winteria* (Sherwood 1981) and the Leptopeltidaceae (Holm and Holm 1977), and the variety of ascus discharge mechanisms within the Rhytismatales (Minter and Cannon 1984).

Previously unknown characters can also come to light and yield data which may be vital in determining relationships. For example, Curry and Kimbrough (1983)

Table 2. Approximate indication of the extent of data on key features required to establish a broadly based system for the Ascomycotina and the size of the orders. Note that none of the larger orders is adequately studied in any feature; + representative taxa studied in detail, − = few or no detailed studies. The system of orders and number of accepted genera and species follows Hawksworth *et al* (1983).

Orders	Characters										Size of orders	
	Anamorph/spermatia	Ascoma structure/ontogeny	Ascus structure/function	Cell wall chemistry	Chromosome numbers	Cytology	Ecology/hosts/substrata	Metabolic products	Phytogeography	Septum structure	Genera	Species
Arthoniales*	−	+	−	−	−	−	+	−	+	−	17	635
Ascosphaerales	+	+	+	−	−	−	+	−	−	−	3	8
Caliciales*	+	+	+	−	−	−	+	+	+	−	35	355
Clavicipitales	+	+	−	−	−	+	+	+	+	−	27	270
Coryneliales	−	−	−	−	−	−	+	−	+	−	5	21
Cyttariales	−	+	−	−	−	−	+	−	+	−	1	10
Diaporthales	+	+	+	−	−	−	+	−	−	−	90	490
Diatrypales	−	+	−	−	−	−	−	−	−	−	22	125
Dothideales*	+	+	+	−	−	−	−	−	−	−	655	6270
Elaphomycetales	−	+	−	−	−	−	+	−	+	−	1	20
Endomycetales	+	+	+	+	−	+	+	+	−	+	69	295
Erysiphales	+	+	+	−	−	−	+	−	+	+	28	105
Eurotiales	+	+	+	+	−	+	+	+	−	+	49	135
Graphidales*	−	+	−	−	−	−	+	+	+	−	30	1655
Gyalectales*	−	+	−	−	−	−	−	−	+	−	12	106
Gymnoascales	+	+	−	−	−	−	+	−	−	−	41	115
Helotiales*	−	+	+	−	−	+	+	−	−	−	345	2000
Hypocreales	+	+	+	−	+	+	+	−	+	−	78	540
Laboulbeniales	+	+	+	−	−	+	+	−	−	−	124	1730
Lecanidiales*	−	+	+	−	−	−	+	−	+	−	17	240
Lecanorales*	−	+	+	+	−	+	+	+	+	−	290	5650
Microascales	+	+	+	−	−	−	−	−	−	−	12	46
Opegraphales*	−	+	+	−	−	−	+	+	+	−	35	885
Ophiostomatales	+	+	+	+	−	+	+	+	−	−	3	85
Ostropales*	−	+	−	−	−	−	+	−	−	−	37	115
Peltigerales*	+	+	+	−	−	+	+	+	+	−	18	590
Pertusariales*	−	+	+	−	−	−	+	+	−	−	6	305
Pezizales	−	+	+	−	−	+	+	−	−	+	144	870
Polystigmatales	−	−	−	−	−	−	−	−	−	−	23	185
Pyrenulales*	−	+	−	−	−	−	+	−	+	−	34	1125
Rhytismatales	+	+	+	−	−	−	+	−	+	−	65	320
Sordariales	−	+	+	+	+	+	+	−	−	+	74	560
Spathulosporales	+	+	+	−	−	+	+	−	+	−	1	5
Sphaeriales*	+	+	+	−	−	+	+	−	+	+	225	1275
Taphrinales	−	+	+	+	−	+	+	−	+	+	9	120
Teloschistales*	+	+	+	−	−	−	+	+	+	−	11	595
Verrucariales*	−	−	−	−	−	−	+	−	−	−	25	710
Percentage studied	46	91	65	16	5	38	86	27	54	19	Total** 2720	28650

* Orders which consist entirely of or include at least some lichen-forming species. ** Totals allow for known taxa not referred to any order.

reported septum types in the apothecia of *Peziza* which were distinct from those in *Neurospora*; such features need to be considered by taxonomists.

Information on secondary metabolites is extremely uneven. Coverage in Graphidales, Lecanorales, Opegraphales, Peltigerales, Pertusariales and Teloschistales is very good with some 4500 species examined (Culberson 1969, 1970; Culberson *et al* 1977), but in the non-lichenized orders only certain groups in the Clavicipitales, Endomycetales and Eurotiales can be viewed as adequately studied (Turner 1971; Turner and Aldridge 1983). Nevertheless, some can be of major interest in other groups, for example carotenoids (Valadon 1976), and novel substances remain to be discovered in families never known to be of chemical interest such as the Xylariaceae (Edwards, in Turner and Aldridge 1983). Gaps in our knowledge of cell-wall chemistry are even larger, but again can be highly significant (Weijman and de Hoog 1975).

Chromosomes are notoriously difficult to study in fungi because of their small size but determination of their numbers could be of immense importance in the delimitation of families and orders. However, techniques for their study do exist (Punithalingam 1983; Sharma and Koul 1984) and much more attention could usefully be directed to this topic.

In all data collection care is also needed in ensuring that the reported features are correctly interpreted. Variations in the iodine reactions of ascal tissues due to poor techniques have been a particular cause of confusion (Nannfeldt 1976).

3.5 *Convergence*

Convergence in characters can lead to particularly heterogeneous groupings based on the convergent feature. Within the Ascomycotina perhaps the best known examples are the development of closed ascoma (cleistothecia) and elongate ascoma (lirellae). The presence of cleistothecia was used to characterize the Plectomycetes in many early systems and persisted into the early 1970s in some works. This feature is, however, now regarded as of little importance and is known to vary within genera and even within single species (von Arx 1973). Nevertheless, while cleistothecial genera are being referred to other groups (*e.g.* Malloch 1981), the data is often inadequate to do this with confidence; this was stressed by Benny and Kimbrough (1980) who consequently retained this class name. Lirellate ascomata are similarly widespread and are to be seen in representatives of several orders including the Dothideales, Graphidales, Lecanorales, Opegraphales and Rhytismatales. Another trend which can be recognized is the production of multi-spored asci, seen in members of the Diaporthales, Diatrypales, Dothideales, Helotiales, Hypocreales, Lecanorales, Ostropales, Pezizales and Verrucariales; in this case comparable results can be achieved by different mechanisms (*i.e.* cut out separately from the protoplast, fragmenting spores, mitotic division, or conidia produced directly from the ascospores). The converse of this, the production of a smaller number of large ascospores can also occur. Filiform ascospores also appear to have repeatedly arisen (Sherwood 1981).

When convergence involves ascus structure it can be a particular source of confusion. Simplification in relation to spore release through ascus deliquescence rather than active discharge can be seen, for example, in several genera of the Xylariaceae (*e.g. Ascotricha*, *Camillea*, *Rhopalostroma*, *Thamnomyces*) where retention in that family is confirmed by the nature of the anamorphs (Samuels and Müller 1980; Hawksworth and Whalley 1985). Asci of lignicolous discomycetes are often also

difficult to interpret, and convergent tendencies in pigmented exciples with a tendency to curve over the discs, and the production of a dark epithecium have been noted (Sherwood 1981). In many cases convergence appears to be paedomorphic, juvenile characters ceasing to develop beyond a particular stage. This phenomenon is particularly evident in relation to ascospore septation, as discussed by Eriksson (1981), but as that is mainly a matter of concern at the generic and specific level it is not pertinent to consider that aspect further here.

3.6 *Delimitation of genera*

In addition to the lack of available information on critical characters in many groups already referred to, discussions on the placement and affinities of particular genera are obscured because the genera themselves are unacceptably heterogeneous. For many years the non-lichenized *Karschia* was included in the concept of *Buellia* and used to encompass a wide range of taxa; it came to occupy a key position in debates on both biological and taxonomic relationships on the assumption that it was homogeneous (*e.g.* Booth 1966; Santesson 1967). However, on careful examination Hafellner (1979) found that species of this "genus" had to be dispersed through 19 genera belonging to at least two orders and a wide range of families. This situation is not atypical of that still existing in many groups of ascomycetes where families and genera have continued to be based on nineteenth century criteria such as ascomatal colour or ascospore colour and septation without regard to ascus structure or centrum type.

3.7 *Undiscovered genera*

Lastly, as with all groups of microfungi, new genera are being recognized at an alarming rate. This results either from a clearer understanding of previously known fungi or more commonly from new collections or isolations. If the assumption that only about one quarter of all fungi have so far been recorded is correct (Hawksworth *et al* 1983), this means that of the order of 8000 genera and 86000 species of ascomycetes remain to be collected and formally described; at the present rate of 25 descriptions of new ascomycete genera per year, our inventory of the genera might not be completed for another three centuries. Whether the numbers are really so gigantuan is doubtful, but what is clear is that as new regions or habitats are carefully explored substantial numbers of new genera and species are still easily discovered today. For example, Döbbeler (1978) found it necessary to describe nine new genera and 62 new species in his survey of pyrenocarpous fungi on bryophytes—a total which continues to rise. The same situation is true of the marine lignicolous fungi; knowledge on these has expanded dramatically over the last few decades, some 180 ascomycetes are now known from such habitats and these not only include numerous new genera and 150 new species, but the new order Spathulosporales and a major family, the Halosphaeriaceae (see Kohlmeyer and Kohlmeyer 1979). Even isolations from soils regularly reveal new genera which are difficult to accommodate in existing orders or families, as is the case with *Bimuria* and *Neotestudina*. In attempts to develop a system for the Ascomycotina, we are consequently working with only a proportion of the pieces which we wish to accommodate into our jig-saw.

4. Towards an acceptable system

Having endeavoured to identify the constraints it is next appropriate to consider how we can proceed towards a generally acceptable system for the Ascomycotina.

4.1 *Users*

Clements and Shear (1931), as noted above, were conscious of the need for a stable system. Repeatedly changing systems tend to bring the science of taxonomy into disrepute, and, as Heywood (1983) has recently stressed, taxonomists need to pay increased regard to the requirements of their users. I think we have to be careful to distinguish between *generalist systems* which we can commend for use by a wide variety of users, and *specialist systems* proposed as hypotheses as a basis for discussion amongst specialists. A generalist system must be relatively stable so that it can be taught to students, incorporated into textbooks, and will be of lasting value to students in that the groups recognized are securely based; it should accurately reflect what is known and hence what is unlikely to be rendered obsolete or considered unsound in the forseeable future. To do more than this is to imply that we are confident of much more than our evidence unequivocably supports, an approach I find unacceptable for general use. In proposing generalist systems which experience shows will be widely adopted by a broad spectrum of users, such as that in the *Dictionary of the Fungi* (Hawksworth *et al* 1983), a not inconsiderable responsibility falls on to the proposers. While this is not to deny the stimulating effect of specialized systems, as it is only through the development and testing of new hypotheses that science can progress, one must also question whether it is wise to encourage the widespread adoption of schemes that are still in a testing phase. Those lecturers trying to keep abreast of the most recent system for the Ascomycotina over the last few decades have had a far from easy task (see table 1) and one can appreciate the sentiment reflected in "Ascomycetes in Disarray" (see p. 1). I feel it is incumbent on specialists proposing new systems to differentiate for their audiences between generalist systems which are soundly based, accepted by most specialists, and merit widespread adoption, and specialist systems which are working hypotheses for debate with specialist colleagues who have yet to be convinced that they merit general use.

The repeated shuffling of names at all ranks brings taxonomy into disrepute. Changes should ideally only be made on the basis of overwhelming new evidence which will make the taxa more meaningful to those who use them. Taxonomists may often work in isolated laboratories, but they can cause confusion through wide areas of pure and applied biology.

A further aspect of stability is that of names. Names above that of family are not subject to the Code and individual authors can vary, for example, ordinal names they wish to use as they see fit. In fact, remarkable restraint in varying ordinal names has been shown, but problems do persist (*e.g.* Sphaeriales vs Xylariales). If ordinal names in particular are to continue to be excluded from the Code, there may be a case for specialist mycologists to meet, debate, and recommend the usage of particular names. Although the names of families come within the remit of the Code, no family name of fungi has yet been accepted for conservation and many mycologists have not applied the existing rules strictly to names in this rank. Cooke and Hawksworth (1970) drew attention to the problem of family names with colloquial German or French

terminations that had received wide acceptance, recommending that these simply be treated as orthographic errors. Proposals to formalize this interpretation (Petersen 1974) were regrettably not accepted and the matter remains of considerable concern to mycologists (Machol 1984).

Many special provisions in the Code have been made for fungi but not all have been adequate and confusions have resulted. The fewer of these that are needed the better (Weresub 1979), and with the changes recently made (Hawksworth 1984) we can now hope to move towards a greater stability in the rules affecting fungi.

4.2 *Data collection*

The problem of uncollected and undescribed species (see p. 14) is not unique to mycology. It has been repeatedly stressed that a major effort to complete the inventory of tropical taxa should be made during the next two decades (Prance 1984); I fully endorse this view. Mycologists should ensure that they take opportunities to collect in the tropics as a part of the major initiatives now being planned (Solbrig and Golley 1983). If proposals to establish an Institute for Tropical Mycology (Subramanian 1982) can be implemented this could make a major contribution to this problem.

Enormous gaps also exist in the information ideally needed on characters of the taxa we do know that can be expected to contribute to the production of a satisfactory scheme for the Ascomycotina (table 2). Substantial progress has been made in the last two decades on ascus structure, centrum types and anamorph connections, but other aspects are scarcely yet explored in more than a handful of groups: septal ultrastructure, cytology, genome similarity, cell-wall composition, enzyme production, and secondary metabolites. Studies on aspects which might yield clues to the evolutionary direction of clines of characters that might be identified are even more meagre. In this last category occurrences on different host groups or substrata (*e.g.* groups confined to mammal dung almost certainly did not originate before mammals), phytogeography with reference to plate tectonics (see Pirozynski and Weresub 1979; Sipman 1983), and co-evolutionary considerations (*e.g.* Hawksworth 1981, 1982; Korf 1983) may be expected to assume increasing importance in future debates.

A reasonably complete data matrix for the whole of the Ascomycotina, together with pertinent ecological and geographical observations, is not likely to be realizable in the forseeable future. Yet what the users of a generalist system require is something that has the highest possible predictive value within the units recognized; this can only be achieved if those units are based on the maximum information obtainable, *i.e.* the objective should be what I will refer to as a *maximum information content system.*

In order to progress towards a maximum information content system I suggest that the most practical way to proceed is to consider multidisciplinary data collection projects on an order or family basis. This method of tackling large groups has already met with considerable success in some large vascular plant groups, such as the Compositae (Heywood *et al* 1977), Leguminosae (Polhill and Raven 1981) and Umbelliferae (Heywood 1971). The major international workshops held on conidial fungi (Kendrick 1971, 1979) also stimulated work on these fungi and led to important agreements on the usage of terms, something also needed in the Ascomycotina (Eriksson 1981; Sipman 1983). I have no doubt that one or more international specialist workshops would be a major impetus in deciding priorities and standardizing terminologies; a limited number of specialist laboratories with complementary

expertise could then generate significant data sets on some presently scarcely studied orders within 5–10 years. However, to determine which orders should be tackled first, and then arrange the funding and co-ordination of work programmes, would require considerable administrative skills. These matters should be addressed at an appropriate international meeting as a matter of some urgency.

Mycologists should also note that a considerable array of computer software is now available for the building up of databases on systematics (Allkin and Bisby 1984), although costs are likely to preclude this option for all but the major economically important groups.

Manpower shortages are also a constraint to data collection. Even where funds are available, it is difficult to find trained taxonomists able to undertake the work required. In view of this limitation, it would be particularly unfortunate if too much energy was dissipated in the construction and debate of hypothetical specialist systems at a time when data collection should be our priority, however academically absorbing such debates can be (Demoulin 1975).

However, by concentrating individually or collaboratively on particular manageable groups, we can expect, albeit it slowly, to progress piece-meal towards the accumulation of the data necessary for system construction.

4.3 *System construction*

If data collection, as suggested above, is concentrated on particular orders or families, we can expect the continuing trend in the recognition of almost certainly monophyletic groups in these ranks to continue. Atypical families or genera will be gradually excluded, and some of these, often previously resting uneasily in widely scattered groups may occasionally be found to form cohesive but hitherto unrecognized families (*e.g.* Odontotremataceae recognized by Sherwood and Hawksworth 1982). Such groups, once identified or refined, tend to be adopted almost immediately by most specialists provided that they are based on an adequate data set. They will also tend to have considerable predictive value as to the characters and behaviour of the groups; *i.e.* they form the units from which a maximum information content system can gradually be built.

I therefore strongly support the view of Eriksson (1981) that a sound and non-speculative route is to be sought through a downward reconstruction of phylogeny, and so our classification, and that the first step in this is the recognition of natural families and orders. In view of the difficulties reviewed earlier, particularly the age of the group, I think that we have to be realistic and not expect to be able to produce a full phylogenetic classification with which we can be confident. However, it is of interest to note that Chadefaud's (*e.g.* Chadefaud 1960, 1973; Chadefaud *et al* 1970) stress on the ancient status of some of the asci found in lichen-forming ascomycetes is now being returned to by an increasing number of specialists. For example, Eriksson (1981) and Hawksworth (1982) independently and following quite different lines both proposed that semifissitunicate asci of the type now seen in the Peltigerales could have given rise to both an ascohymenial unitunicate line and an ascolocular bitunicate line. However, I do not feel we yet have enough information to reflect any such considerations in a generalist system. For the present we should therefore concentrate on making a scheme of monophyletic orders and families available to the wider users of taxonomy that we serve, reserving such speculative specialist systems for peer review and specialist debate.

In view of our limitations of appropriately skilled manpower and the vast size of the Ascomycotina, recognizing that no one mycologist will ever be able to embrace the whole group, I can see no alternative but to a collaborative effort in building up our system of orders and families. Even the multidisciplinary approach to data collection in individual orders or families will not of course overcome the perhaps more daunting synthesis phase in the production of a maximum information content generalist system. One way in which to implement such a collaboration in synthesis has already been introduced by Eriksson (1983) who is starting to issue modified schemes on an annual basis, taking note not only of the published work but also the specialists writing in to suggest refinements. He deserves the strongest support of all mycologists working with ascomycetes in this task. Indeed, should he eventually relinquish this role, it would be necessary for some other individual or group to assume this mantle. It appears to be the only course towards an acceptable system, towards order out of chaos, available to us at this time.

References

Ainsworth G C, James P W and Hawksworth D L 1971 *Ainsworth and Bisby's dictionary of the fungi*, 6th edn (Kew: Commonwealth Mycological Institute)

Ainsworth G C, Sparrow F K and Sussman A S (eds) 1973 *The fungi: an advanced treatise*, (New York, London: Academic Press) vol. 4A

Allkin R and Bisby F A (eds) 1984 *Databases in systematics* (London: Academic Press)

von Arx J A 1973 Ostiolate and non-ostiolate Pyrenomycetes; *Proc. K. Ned. Akad. Wet.* C 76 289–296

von Arx J A 1979 Ascomycetes as fungi imperfecti; in *The whole fungus* (ed.) B Kendrick (Ottawa: National Museums of Canada) 201–213

von Arx J A 1981 *The genera of fungi sporulating in pure culture*, 3rd edn (Vaduz: Cramer)

von Arx J A, Walt J P van der and Liebenberg N V D M 1982 The classification of *Taphrina* and other fungi with yeast-like cultural states; *Mycologia* **74** 285–296

von Arx J A and Müller E 1954 Die Gattungen der amerosporen Pyrenomyceten; *Beitr. Kryptogamenflora Schweiz* **1** 1–434

von Arx J A and Müller E 1975 A re-evaluation of the bitunicate ascomycetes with keys to families and genera; *Stud. Mycol.* **9** 1–159

Barr M E 1976 Perspectives in the Ascomycotina; *Mem. N.Y. Bot. Gdn* **28** 1–8

Barr M E 1979 A classification of Loculoascomycetes; *Mycologia* **71** 935–957

Barr M E 1983 The ascomycete connection; *Mycologia* **75** 1–13

Bellemère A 1968 "1967" Contribution a l'étude de développement de l'apothécie chez les discomycètes inoperculés; *Bull. Soc. Mycol. Fr.* **83** 395–931

Bellemère A 1971 Les asques et des apothécies des discomycètes bituniqués; *Ann. Sci. Nat., Bot. Biol. Vég.* **12** 429–464

Benny G L and Kimbrough J W 1980 A synopsis of the orders and families of Plectomycetes with keys to genera; *Mycotaxon* **12** 1–91

Bessey E A 1935 *A text-book of mycology* (Philadelphia: Blakiston's)

Booth C 1966 Fruit bodies in ascomycetes; in *The fungi: an advanced treatise* (eds) G C Ainsworth and A S Sussman (New York etc: Academic Press) **2** 133–150

Cain R F 1972 Evolution of the fungi; *Mycologia* **60** 1–14

Cannon P F, Hawksworth D L and Sherwood-Pike M A 1985 *The British Ascomycotina. An annotated checklist* (Kew: Commonwealth Mycological Institute)

Chadefaud M 1960 Les vegetaux non vasculaires (Cryptogamie); *Traité de botanique systématique* (eds) M Chadefaud and L Emberger (Paris: Masson) vol. I

Chadefaud M 1973 Les asques et la systématique des Ascomycètes; *Bull. Soc. Mycol. Fr.* **89** 127–170

Chadefaud M 1982a Les principaux types d'ascocarpes: leur organisation et leur evolution; *Cryptog., Mycol.* **3** 1–9

Chadefaud M 1982b Les principaux types d'ascocarpes: leur organisation et leur evolution; Deuxième partie: Les discocarpes; *Cryptog., Mycol.* **3** 103–144

Chadefaud M, Letrouit-Galinou M-A and Janex-Favre M-C 1970 "1969" Sur l'origine phylogénétique et l'evolution des Ascomycètes des lichens; *Mém. Soc. bot. Fr.* 1968 79–111

Chesters C G C 1938 Studies on British pyrenomycetes II. A comparative study of *Melanomma pulvis-pyrius* (Pers.) Fuckel, *Melanomma fuscidulum* Sacc. and *Thyridaria rubro-notata* (B. & Br.) Sacc.; *Trans. Br. Mycol. Soc.* **22** 116–150

Clements F E 1909 *The genera of fungi* (Minneapolis: Wilson)

Clements F E and Shear C L 1931 *The genera of fungi* (New York: Wilson)

Cooke W B and Hawksworth D L 1970 A preliminary list of the families proposed for fungi (including the lichens); *Mycol. Pap.* **121** 1–86

Culberson C F 1969 *Chemical and botanical guide to lichen products* (Chapel Hill: University of North Carolina Press)

Culberson C F 1970 Supplement to "Chemical and botanical guide to lichen products"; *Bryologist* **73** 177–377

Culberson C F, Culberson W L and Johnson A 1977 *Second supplement to "Chemical and Botanical guide to lichen products"* (St. Louis: American Bryological and Lichenological Society)

Curry K J and Kimbrough J W 1983 Septal structures in apothecial tissues of the Pezizaceae (Pezizales, Ascomycetes); *Mycologia* **75** 781–794

Demoulin V 1975 The origin of Ascomycetes and Basidiomycetes. The case for a red algal ancestry; *Bot. Rev.* **40** 315–345

Döbbeler P 1978 Moosbewohnende Ascomyceten I. Die Pyrenocarpen, den Gametophyten besiedelnden Arten; *Mitt. Bot. Staatssamml. Muencho* **14** 1–360

Eriksson O 1981 The families of bitunicate ascomycetes; *Opera Bot.* **60** 1–220.

Eriksson O 1982 Outline of the ascomycetes – 1982; *Mycotaxon* **15** 203–248

Eriksson O 1983 Outline of the ascomycetes – 1983; *Systema Ascom.* **2** 1–37

Gäumann E 1964 *Die Pilze. Grundzüge ihrer Entwichlungsgeschichte und Morphologie*, 2nd edn (Basel and Stuttgart: Birkäuser)

Hafellner J 1979 *Karschia.* Revision einer Sammelgattung an der Grenze von lichenisierten und nichtlichenisierten Ascomyceten; *Beih. Nova Hedwigia* **62** 1–248

Hale M E 1967 *The biology of lichens* (London: Arnold)

Hansford C G 1946 The foliicolous Ascomycetes their parasites and associated fungi; *Mycol. Pap.* **15** 1–240

Hawksworth D L 1978 The taxonomy of lichen-forming fungi: reflections on some fundamental problems; in *Essays in plant taxonomy* (ed.) H E Street (London etc: Academic Press) 212–243

Hawksworth D L 1981 A survey of the fungicolous conidial fungi; in *The biology of conidial fungi* (eds) G T Cole and B Kendrick (New York etc: Academic Press) **1** 171–244

Hawksworth D L 1982 Co-evolution and the detection of ancestry in lichens; *J. Hattori Bot. Lab.* **52** 323–329

Hawksworth D L 1984 Recent changes in the international rules affecting the nomenclature of fungi; *Microbiol. Sci.* **1** 18–21

Hawksworth D L, Sutton B C and Ainsworth G C 1983 *Ainsworth and Bisby's dictionary of the fungi*, 7th edn (Kew: Commonwealth Mycological Institute)

Hawksworth D L and Whalley A J S 1985 A new species of *Rhopalostroma* with a *Nodulisporium* anamorph from Thailand; *Trans. Br. Mycol. Soc.* **84** (in press)

Henssen A 1963 Eine Revision der Flectenfamilien Lichinaceae und Ephebaceae; *Symb. Bot. Upsal.* **18**(1) 1–123.

Henssen A and Jahns H M 1973 "1974" *Lichenes. Eine Einführung in die Flechtenkunde* (Stuttgart: Thieme)

Heywood V H (ed.) 1971 *The biology and chemistry of the Umbelliferae* (London and New York: Academic Press)

Heywood V H 1983 The mythology of taxonomy; *Trans. Bot. Soc. Edinburgh* **44** 79–94

Heywood V H, Harborne J B and Turner B L (eds) 1977 *The biology and chemistry of the Compositae*; (London: Academic Press) 2 vols

Holm L and Holm K 1977 A study of the Leptopeltidaceae; *Bot. Not.* **130** 215–229

Honegger R 1978a *Licht- und elektronenoptische Untersuchungen an Flechten-Asci von* Lecanora-*typ* (Basel: Universitat)

Honegger R 1978b The ascus apex in lichenized fungi I. The *Lecanora*, *Peltigera*- and *Teloschistes*-types; *Lichenologist* **10** 47–67

Honegger R 1978c Ascocarpontogenie, Ascusstruktur und -funktion bei Vertretern der Gattung *Rhizocarpon; Ber. Dtsch. Bot. Ges.* **91** 579–594
Honegger R 1980 The ascus apex in lichenized fungi II. The *Rhizocarpon*-type; *Lichenologist* **12** 157–171
Honegger R 1982a Ascus structure and function, ascospore delimitation, and phycobiont cell wall types associated with the Lecanorales (lichenized ascomycetes); *J. Hattori Bot. Lab.* **52** 417–429
Honegger R 1982b The ascus apex in lichenized-fungi III. The *Pertusaria*-type; *Lichenologist* **14** 205–217
Honegger R 1983 The ascus apex in lichenized fungi IV. *Baeomyces* and *Icmadophila* in comparison with *Cladonia* (Lecanorales) and the non-lichenized *Leotia* (Helotiales); *Lichenologist* **15** 57–71
Janex M-C 1971 Recherches sur l'ontogénie, l'organisation et les asques de quelques pyrènolichens; *Rev. Bryol. Lichénol.* **37** 421–650
Kendrick W B (ed) 1971 *Taxonomy of fungi imperfecti* (Toronto: University Press)
Kendrick W B (ed.) 1979 *The whole fungus*, (Ottawa: National Museums of Canada) 2 vols
Kohlmeyer J and Kohlmeyer E 1979 *Marine mycology. The higher fungi* (New York: Academic Press)
Korf R P 1983 *Cyttaria* (Cyttariales): coevolution with *Nothofagus*, and evolutionary relationship to the Boedijnopezizeae (Pezizales, Sarcoscyphaceae); *Aust. J. Bot., Suppl.* **10** 77–87
Kreisel H 1969 *Grundzüge eines natürlichen Systems der Pilze* (Jena: Gustav Fischer)
Letrouit-Galinou M-A 1967 "1966" Recherches sur l'ontogénie et l'anatomie comparative des apothécies de quelques Discolichens; *Rev. Bryol. Lichénol.* **34** 413–588
Letrouit-Galinou M-A 1969 "1968" The apothecia of the discolichens; *Bryologist* **71** 297–327
Letrouit-Galinou M-A 1973 Les asques des lichens et le type archaeascé; *Bryologist* **76** 30–47
Lindsay W L 1869 Observations on new lichenicolous micro-fungi; *Trans. R. Soc. Edinburgh* **25** 513–555
Locquin M V 1984 *Mycologie générale et structurale* (Paris, etc.: Masson)
Luttrell E S 1951 Taxonomy of the pyrenomycetes; *Univ. Mo. Stud.* **24** 1–120 [reprinted as *Bibl. Mycol.* **6** 1967]
Luttrell E S 1955 The ascostromatic Ascomycetes; *Mycologia* **47** 511–531
Machol R E 1984 Leave the Code alone; *Taxon* **33** 532–533
Malloch D W 1981 The plectomycete centrum; in *Ascomycete systematics: The Luttrellian concept* (ed.) D R Reynolds 73–91 (New York: Springer Verlag)
Miller J H 1949 A revision of the classification of the Ascomycetes with special emphasis on the Pyrenomycetes; *Mycologia* **41** 99–127
Minter D W and Cannon P F 1984 Ascospore discharge in some members of the Rhytismataceae; *Trans. Br. Mycol. Soc.* **83** 65–92
Müller E and von Arx J A 1950 Einige Aspekte zur Systematik pseudosphärialer Ascomyceten; *Ber. Schweiz. Bot. Ges.* **60** 329–397
Müller E and von Arx J A 1962 Die Gattungen der didymosporen Pyrenomyceten; *Beitr. Kryptogamenflora Schweiz* **11** 1–922
Nannfeldt J A 1932 Studien über die Morphologie und systematik der nich-lichenisierten inoperculaten Discomyceten; *Nova Acta R. Soc. Scient. Upsal.* IV **8** 1–368
Nannfeldt J A 1976 Iodine reactions in ascus plugs and their taxonomic significance; *Trans. Br. Mycol. Soc.* **67** 283–287
Parguey-Leduc A 1966 Recherches sur l'ontogenie et l'anatomie comparée des ascocarpes des pyrenomycetes ascoloculaires; *Ann. Sci. Nat., Bot.* XII **7** 505–690
Petersen R M 1974 Proposals for treatment of fungus family names with vernacular terminations; *Taxon* **23** 654–658
Pirozynski K A and Weresub L K 1979 A biogeographic view of the history of ascomycetes and the development of their pleomorphism; in *The whole fungus* (ed.) B Kendrick (Ottawa: National Museums of Canada) **1** 93–123
Poelt J 1974 "1973" Classification; in *The lichens* (eds) V Ahmadjian and M E Hale (New York and London: Academic Press) 599–632
Polhill R M and Raven P H 1981 *Advances in legume systematics* (Kew: Royal Botanic Gardens) 2 vols
Prance G T 1984 Completing the inventory; in *Current concepts in plant taxonomy* (eds) V H Heywood and D M Moore (London: Academic Press) 365–396
Punithalingam E 1983 The nuclei of *Macrophomina phaseolina* (Tassi) Goid.; *Nova Hedwigia* **38** 339–367
Reynolds D R (ed.) 1981 *Ascomycete systematics. The Luttrellian concept* (New York: Springer Verlag)
Richardson D H S 1970 Ascus and ascocarp structure in lichens; *Lichenologist* **4** 350–361
Samuels G J and Müller E 1980 Life-history studies of Brazilian ascomycetes 8; *Sydowia* **33** 274–281

Santesson R 1949 *Dolichocarpus* and *Xanthopeltis*, two new lichen genera from Chile; *Sven. Bot. Tidskr.* **43** 547–567

Santesson R 1952 Foliicolous lichens I. A revision of the taxonomy of the obligately foliicolous lichenized fungi; *Symb. Bot. Upsal.* **12**(1) 1–590

Santesson R 1953 The new systematics of lichenized fungi; in *Proc. Seventh Int. Bot. Cong., Stockholm 1950* (eds) H Osvald and E Åberg (Stockholm: Almqvist and Wiksell) 809–810

Santesson R 1967 On taxonomical and biological relations between lichens and non-lichenized fungi; *Bot. Not.* **120** 497–498

Sharma A K and Koul A K 1984 Chromosomal variability in *Phyllactina* (Erysiphaceae); *Trans. Br. Mycol. Soc.* **82** 355–359

Sheard J W 1977 Palaeogeography, chemistry and taxonomy of the lichenized ascomycetes *Dimeleana* and *Thamnolia*; *Bryologist* **80** 100–118

Sherwood M A 1981 Convergent evolution in discomycetes from bark and wood; *Bot. J. Linn. Soc.* **82** 15–34

Sherwood-Pike M A 1985 *Pelicothallus* Dilcher, an overlooked fossil lichen; *Lichenologist* **17** 114–115

Sherwood M A and Hawksworth D L 1982 Two new families in the Ascomycotina; *Mycotaxon* **16** 262–264

Sipman H 1983 A monograph of the lichen family Megalosporaceae; *Bibltheca lich., Vaduz* **18** 1–241

Sivanesan A 1984 *The bitunicate ascomycetes and their anamorphs* (Vaduz: Cramer)

Solbrig O T and Golley F B 1983 A decade of the tropics; *Int. Biol. Spec. Issue* **2** 1–15

Subramanian C V 1982 Tropical mycology: future needs and development; *Cur. Sci.* **51** 321–325

Tehler 1983 The genera *Dirina* and *Roccellina* (Roccellaceae); *Opera Bot.* **70** 1–86

Turner W B 1971 *Fungal metabolites* (London and New York: Academic Press)

Turner W B and Aldridge D C 1983 *Fungal metabolites II* (London: Academic Press)

Valadon L R G 1976 Carotenoids as additional taxonomic characters in fungi: a review; *Trans. Br. Mycol. Soc.* **67** 1–15

Vězda A 1968 Taxonomische Revision der Gattung *Thelopsis* Nyl. (lichenisierte Fungi); *Folia Geobot. Phytotax. Praha* **4** 363–406

Watson W 1929 The classification of lichens; *New Phytol.* **28** 1–36, 85–116

Wehmeyer L E 1975 The pyrenomycetous fungi; *Mycol. Mem.* **6** 1–250

Weijman A C M and de Hoog G S 1975 On the subdivision of *Ceratocystis*; *Antonie van Leeuwenhoek* **41** 353–360

Weresub L K 1979 Mycological nomenclature: reflections on its future in the light of its past; *Sydowia Beih.* **8** 416–431

Zahlbruckner A 1926 Lichens (Flechten). B. Spezieller Teil; *Nat. Pflanzenfam.* **8** 61–270

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 341–345.
© Printed in India.

On *Achaetomium* and a new genus *Subramaniula* (Ascomycota)

J A VON ARX

Centraalbureau voor Schimmelcultures, Baarn, The Netherlands

Abstract. The genera *Chaetomium* and *Achaetomium* cannot be distinguished by the presence or absence of ascomatal hairs, but only by a combination of other characters. Several *Achaetomium* species are transferred to *Chaetomium*. A new genus *Subramaniula* is proposed for *Achaetomium thielavioides*.

Keywords. Fungal taxonomy; *Chaetomium*; *Achaetomium*; *Subramaniula*.

1. Introduction

In the course of a revision of *Chaetomium* and its relatives (von Arx *et al* 1984), the species of *Achaetomium* were also considered. *Achaetomium* was described by Rai *et al* (1964) for *A. globosum* Rai & Tewari (type species), *A. luteum* Rai & Tewari and *A. strumarium* Rai *et al*, all isolated from soil in India. Several more species have since been added. Chowdhery (1980) provided a key and short descriptions of 12 Indian species. Udagawa and Sugiyama (1982) described two additional species, isolated from soil collected in Nepal. Von Arx *et al* (1978) described a coprophilous species collected in Delhi. The only non Indian species is *A. cristalliferum* Faurel & Locquin-Linard (Locquin-Linard 1980), isolated from soil collected in Egypt.

Achaetomium was originally distinguished from *Chaetomium* by its smooth ascomata, a contrast to the hairs or setae which ornament the ascomata of the latter genus. A study of *A. globosum* and other species shows, that this distinction is not tenable, because the ascomata are covered with delicate, pale, but distinct hairs or hyphae. Several *Chaetomium* species, on the other hand, have only short, indistinct or delicate ascomatal hairs, *e.g. Ch. cruentum* Ames, *Ch. umbonatum* Brewer, *Ch. depectivum* Malloch or *Ch. vitellinum* Carter.

Some other characters, however, proved to be useful for distinguishing *Achaetomium*, in a restricted sense, from *Chaetomium*. The colonies of typical *Achaetomium* species at 28 C on corn meal agar show a daily growth rate of more than 8 mm, while typical *Chaetomium* species grow slower. The ascomata in *Achaetomium* have a thick, usually dark wall, composed of relatively small hyphal cells (textura intricata in surface view). The ascomatal wall of *Chaetomium* species is usually thinner and paler or it is composed of angular cells (textura angularis). In *Achaetomium* the asci are narrow cylindrical. Only a few *Chaetomium* species have such asci, *e.g. Ch. brasiliense* Batista & Pontual and *Ch. senegalense* Ames. The ascospores in typical *Achaetomium* species are opaque or greenish black when mature. No *Chaetomium* species have such darkly pigmented ascospores. This character, therefore, is the easiest to separate the two genera. *Achaetomium* has to be characterized as follows:

ACHAETOMIUM Rai & Tewari
Colonies expanding, with a pale aerial mycelium and a dark reverse; ascomata superficial, discrete or aggregated, spherical or ovate, ostiolate, covered with delicate, pale, punctulate or verrucose hairs; ascomatal wall thick, dark, composed of several layers of small hyphal cells, textura intricata in surface view; asci narrow cylindrical, 8–spored, evanescent, without distinct paraphyses; ascospores 1-celled, unsheathed, oblate, spherical, ovate or ellipsoidal, opaque or greenish black when mature, with a germ pore; anamorphs absent.

2. Accepted species

Achaetomium globosum Rai & Tewari
Achaetomium globosum Rai & Tewari – Can. J. Bot. 42: 693. 1964.
Thielaviella octospora Natarajan – Proc. Ind. Nat. Sci. Acad., B, 37: 128. 1971.
Chaetomium spinigerum Sörgel ex Seth – Beih. Nova Hedwigia 37: 102. 1972.
Achaetomium marinum Chowdhery & Rai – Nova Hedwigia 32: 225. 1980.

Colonies with a daily growth rate of 10–11 mm at 28 C, with a pale aerial mycelium and a dark reddish-brown reverse; ascomata superficial, tomentose, yellow-green in reflected light, spherical or obovate, ostiolate, 150–280 μm in diam, with a thick, brown wall which is of textura intricata in surface view; ascomatal hairs long, flexuous or undulate, pale, punctulate, 1·5–2·5 μm broad; asci cylindrical, 8-spored, evanescent, 70–82 × 9–13 μm; ascospores uniseriate, spherical or slightly oblate, opaque when mature, 10–13 × 8–11 μm, with a distinct germ pore.

Several specimens were studied, among which are types of *A. globosum* (CBS 332·67) and *Ch. spinigerum* (IMI 73515). *A. marinum* was distinguished from *A. globosum* by larger ascomata, as these are in the type of *Ch. spinigerum*. The species has been collected in China, India and Pakistan.

Achaetomium luteum Rai & Tewari
Achaetomium luteum Rai & Tewari – Can. J. Bot. 42: 694. 1964.
Achaetomium brevisemum Chowdhery & Rai – Nova Hedwigia 32: 225. 1980.

Colonies with a daily growth rate of 8–9 mm at 28 C, with a pale, often greenish aerial mycelium, a dark, grey green reverse and often with yellow or red exudates; ascomata superficial, tomentose, pale grey in reflected light, spherical or ovate, ostiolate, 110–200 μm in diam, with a thick, brown wall which is of textura intricata in surface view; covering hyphae flexuous or undulate, unbranched, pale, 1·5–2·5 μm broad, verrucose or covered with acicular crystals; asci cylindrical, 8–spored, evanescent, 45–60 × 6–8 μm, without distinct paraphyses; ascospores uniseriate, ellipsoidal or ovate, with attenuated and rounded ends, opaque or greenish black when mature, 8–11·5 × 5·5–7·5 μm, with an apical germ pore.

Several specimens were studied, among which is a subculture of the type (CBS 618·68). The type of *A. brevisemum* was not available. This species was distinguished from *A. luteum* by smaller ascomata. The strains NHL 2853 and CBS 715·74 also have relatively small ascomata and red exudates have been observed in the latter. *A. luteum* is known from Australia, India, Japan, Pakistan and Kenya.

Achaetomium strumarium Rai et al.
Achaetomium strumarium Rai et al. – Can. J. Bot. 42: 694. 1964.

Chaetomium spinulosum Sörgel ex Seth – Beih. Nova Hedwigia 37: 103. 1972.
Chaetomium sulphureum Sörgel ex Seth – Beih. Nova Hedwigia 37: 108. 1972.
Achaetomium cristalliferum Faurel & Locquin-Linard – Cryptog. Mycol. 1: 235. 1980.
? *Achaetomium macrocarpus* Rai & Chowdhery – Kavaka 1: 32. 1973.

Colonies with a daily growth rate of 10–12 mm at 28 C and a pale, white or yellow aerial mycelium, often with a red exudate; reverse dark reddish brown; ascomata superficial, often clustered, tomentose, yellow green or reddish in reflected light, spherical, ovate or apically conical, ostiolate, 150–270 μm, with a dark, thick wall of small cells, textura intricata in surface view; ascomatal hyphae flexuous or wavy, delicate, pale, verrucose or covered with acicular crystals, 1·5–2·5 μm broad; asci cylindrical, 8-spored, evanescent, 60–90 × 8–11 μm; ascospores uniseriate, ellipsoidal or ovate, attenuated and rounded at the ends, opaque or dark brown when mature, 10–13 × 6–8 μm, with an apical germ pore.

Several specimens were studied, among which were subcultures of the types of *A. strumarium* (CBS 333·67) and *A. cristalliferum* (CBS 770·81), and the type specimens of *Chaetomium spinulosum* (IMI 73517) and *Ch. sulphureum* (IMI 73518). In the latter the ascomata have a diameter of more than 240 μm and are covered with yellow hyphae, and the ascospores measure 11–14 × 7–8 μm. These characteristics correspond with the description of *A. macrocarpus*, which was not available for comparison. The cultures of *A. cristalliferum* now are sterile. *A. strumarium* occurs on soil and plant debris and was collected in India, Pakistan, Namibia, South Africa, Algeria, Israel and Spain (Canary Islands).

3. Excluded and not examined species

Achaetomium fusisporus Rai & Chowdhery – J. Indian bot. Soc. 52: 310. 1973.
The type was not available. From the description the fungus may be identical with *Chaetomium vitellinum* Carter – Mycologia 75: 531. 1983 (nec *Chaetomium fusisporum* G. Smith).

Achaetomium hamadae Udagawa – Trans. mycol. Soc. Japan 23: 287. 1982.
The type strain NHL 2910 was studied. The ascomata have a pale, reddish wall, covered with delicate, thin, brown hairs with a swollen basal cell. The ascospores are irregular in shape and measure 11–13 × 7–8 μm, with an apical germ pore. The combination CHAETOMIUM HAMADAE (Udagawa) comb. nov. is proposed.

Achaetomium macrosporum Rai et al. – Indian Phytopathol. 23: 54. 1970.
The type was not available. From the description the fungus is rather similar to *Chaetomium vitellinum* Carter.

Achaetomium indicum Kulshrestha et al – Acta bot. Indica 5: 16. 1977.
The type is not present at ITCC and probably is lost.

Achaetomium indicum Rai & Chowdhery – Curr. Sci. 47: 23. 1978.
This species name is a later homonym of *A. indicum* Kulshrestha et al and was renamed by Locquin-Linard (1980) as *Achaetomium raii*. The type could not be examined. The species is described as having ellipsoidal, 19–25 × 10–12 μm ascospores with 2 germ pores.

Achaetomium nepalense Udagawa & Sugiyama – Rep. Crypt. Study in Nepal, Misc. Publ. Nat. Sci. Mus. Tokyo p. 11. 1982.
A subculture of the type was studied (NHL 2895). The fungus is related to *Chaetomium murorum* Corda, from which it can be distinguished mainly by the nearly smooth ascomata. The name CHAETOMIUM NEPALENSE (Udagawa and Sugiyama) comb. nov. is proposed.

Achaetomium purpurascens Udagawa and Sugiyama – Rep. Crypt. Study in Nepal, Misc. Publ. Nat. Sci. Mus. Tokyo p. 13. 1982.
A subculture of the type was studied (NHL 2896). The species is characterized by relatively large ascospores with 2 germ pores. It is related to *Chaetomium aureum* Chivers, which has similar, but smaller ascospores. Both species produce on cornmeal agar a red exudate and relatively small ascomata. The name CHAETOMIUM PURPURASCENS (Udagawa and Sugiyama) comb. nov. is proposed.

Achaetomium sphaerocarpus Rai & Chowdhery – Kavaka 1: 29. 1973.
The type was not available.

Achaetomium sulphureus Rai & Chowdhery – J. Indian bot. Soc. 52: 310. 1973.
The type was not available.

Achaetomium thielavioides v. Arx, Mukerji and Singh – Persoonia 10: 144. 1978.
This fungus is related to some species of *Thielavia* and also shows affinities to *Chaetomium* and *Podospora*. It differs from these genera by urniform, pale ascomata with a translucent wall and a wide, apical opening, surrounded by a colourless collar. *A. thielavioides* may be classified in a separate genus, which is named *Subramaniula* in honour of Prof. C V Subramanian.

SUBRAMANIULA gen. nov.
Ascomata superficialia, urniformia vel lageniformia, apicaliter lato perforata, hyalina vel pallida, glabra, translucida; paries textura epidermoidea vel angulata; asci globosi vel obovati, sessiles, unitunicati, 8-spori, evanescentes; ascosporae ellipsoideae vel fusiformes, continuae, glabrae, pigmentatae, poro germinationes praeditae.

Species typica: *Achaetomium thielavioides* v. Arx, Mukerji and Singh

SUBRAMANIULA THIELAVIOIDES (v. Arx, Mukerji and Singh) comb. nov.
Basionym: Achaetomium thielavioides v. Arx, Mukerji and Singh – Persoonia 10: 144. 1978.
The following description is based on a fresh isolate from dung, collected in 1977 in Delhi (CBS 560·84).
Colonies on cornmeal agar at 28 C in dark with a daily growth rate of 5–6 mm, with a white, floccose aerial mycelium; reverse unchanged; ascomata maturing within 5–6 days, formed from a coil on an aerial hypha and embedded in the mycelium, ovate when young, urniform when mature, with a thin wall of unpigmented, elongate or angular, flattened, 7–12 μm cells (textura epidermoidea or angularis), smooth, translucent, 130–200 μm in diameter, apical opening 50–90 μm wide, surrounded by a collar of hyaline, thin-walled cells; asci in small numbers, often only 5–10, obovate, pyriform or nearly spherical, 8-spored, very evanescent, surrounded by some filaments when young; ascospores broadly fusiform, thick-walled, smooth, brown when mature, 23–27 × 13–17 μm, with a distinct, 1·5–2·5 μm wide, subapical-lateral germ pore.

Achaetomium uniapiculatum Rai & Chowdhery – Curr. Sci. 40: 412. 1971.
The type was not available. From the description, the fungus is identical to *Chaetomium amygdalisporum* Udagawa and Muroi – Trans. mycol. Soc. Japan 22: 13. 1981, and to *Ch. subapiculatum* Gopal & Lodha – Trans. mycol. Soc. Japan 23: 261. 1982, of which subcultures of the types could be studied (NHL 2874; CBS 519·80). The combination CHAETOMIUM UNIAPICULATUM (Rai and Chowdhery) comb. nov. is proposed (Basionym: Cursif Rai and Chowdhery – l.c.). The species is related to *Ch. senegalense* Ames, which also has cylindrical asci and delicate, pale ascomatal hairs, but smaller ascospores.

Acknowledgement

Mr K Seifert is acknowledged for his comments on the manuscript.

References

Arx J A von, Dreyfuss M and Müller E 1984 A revaluation of Chaetomium and the Chaetomiaceae; *Persoonia* **12** 169-179

Arx J A von, Mukerji K G and Singh N 1978 A new coprophilous ascomycete from India; *Persoonia* **10** 144-146

Chowdhery H J 1980 The genus *Achaetomium; Nova Hedwigia* **32** 473–482

Locquin-Linard M 1980 *Achaetomium cristalliferum* (Achaetomiaceae); *Crypt. Mycol.* **1** 235-240

Rai J N, Tewari J P and Mukerji K G 1964 *Achaetomium* a new genus of Ascomycetes; *Can. J. Bot.* **42** 693-697

Udagawa S and Sugiyama Y 1982 Reports on the Cryptogamic Study in Nepal; *Misc. Publ. Nat. Sci. Mus. Tokyo* p. 11-46

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 347–354.
© Printed in India.

Meliolina subramanianii sp. nov.

S J HUGHES and K A PIROZYNSKI*

Biosystematics Research Institute, Agriculture Canada, Ottawa, Ontario K1A 0C6, Canada

*Paleobiology Division, National Museums of Canada (NMNS), Ottawa, Ontario K1A 0M8, Canada

Abstract. A foliicolous fungus occurring on *Syzygium* in the Nilgiris and previously recorded as *Meliolina mollis* (Berk. & Br.) v. Höhn. is redescribed as *M. subramanianii* sp. nov.

Keywords. Fungi; Ascomycetes; *Meliolina; Syzygium.*

The road from Ootacamund, crowning the Nilgiri Hills, to Gudalur on the northern slopes of Wynaad, winds through a high, undulating plateau before descending a steep embankment, in sharp twists and turns, on its way to Mysore. Once lushly forested the Wynaad-Nilgiri countryside bears marks of the climatic depredations of the Pleistocene, the long presence of man, and of his more recent ritual—the afternoon tea. Much of the primary forest has been replaced by heavily grazed, fire-prone shrubby savanna and, since 1836, by plantations of exquisite tea. Only in deeper, more sheltered and moister gullies and depressions remain isolated pockets of indigenous vegetation attesting to the former arborescent glory of the high Western Ghats. Here fungi too, as if seeking refuge, appear to have congregated in the remaining patches of the original vegetation.

The world knows much about these fungi, which often bear exotic names derived from Sanskrit, because Wynaad–Nilgiri, for the past thirty years, has been the favourite stomping ground of C V Subramanian. The fungi are certainly exotic, in aspect as well as name, but only now are we beginning to recognize the extent of their eccentricity: for the ecologically inflexible pockets of tropical, evergreen "shola" forest contain relicts of a different world and of a different era—the Cretaceous Gondwanaland.

In this paper we record one such relict, a fungus collected in Wynaad–Nilgiri at an altitude of about 2000 m along the Mysore road near Gudalur. The fungus was originally recorded as *Meliolina mollis* (Berk. & Br.) v. Höhn. (Pirozynski 1974) but now, following a more critical study, it is redescribed as *M. subramanianii* sp. nov.

Meliolina subramanianii sp. nov.

Coloniae hypophyllae, atrae, dense velutinae, plus minusve circulares, ad 14 mm diam. Mycelium superficiare ex hyphis radiantibus, levibus, ramosis, brunneis vel atrobrunneis, 7·2–9 μm cr. compositum. Stomatopodia lateralia, dispersa, plerumque sessilia interdum breviter stipitata, subglobulosa vel ellipsoidea vel obovoidea, recta vel flexa, ad 29 μm long., 9–12 μm lat., unicellularia, interdum hyphas laterales gerentia. Hyphae immersae per stomata ingredientes, dein intercellulariter extensae.

Phialophora numerosa, dense aggregata, setiformia, erecta, in hyphis superficiaribus portata, ad 370 μm long., 1–4(–5) dichotome ramosa, arborescentia, basim versus atrobrunnea, crasso-tunicata, 9–11 μm cr., apicem versus angustata, 5–6·5 μm cr., brunnea et tenui-tunicata: rami in phialide terminantes. Phialides anguste obclavatae vel plus minusve subulatae, 34–48 μm long., basim versus pallide brunneae et 5·4–8 μm lat., apicem versus subhyalinae et 2·3–2·7 μm lat., collarioque praeditae. Phialoconidia cuneiformia, hyalina, 4–5 × 2·1–2·5 μm.

Perithecia atra, subglobosa ad 450 μm lat., ad 350 μm alt., ostiolata, setis lateralibus simplicibus; plerumque rectis, septatis, atrobrunneis, ad 330 μm long., basim versus 7–9 μm cr., apicem versus 4–7 μm cr. Paraphyses conspicuae, simplices vel basim versus 1–ramosae, hyalinae, ad 190 μm long., septatae (cellulis 27–48 μm long., 5–7·2(–9) μm cr.), ad apicem rotundatae. Asci clavati vel anguste obovoidei, 160–170 × 50–55 μm, (4–)8-spori.

Ascosporae ellipsoideae vel subcylindricae, utrinque subpapillatae vel papillatae, 3–septatae, ad septa constrictae, brunneae vel atrobrunneae sed papillis polaribus subhyalinis vel pallide brunneis, 50–70 × 18–23·4 μm. Sub papilla apicali basalique disco tenui atrobrunneo acetabuliformi 7–8·5 μm lat. aliquando aspectu annuli atrobrunnei. Cellulae polares zona subhyalina, tenuitunicata, angusta (circa 1 μm) sub disco atrobrunneo et prope septa praeditae.

In foliis vivis *Syzygii ?montani* (Wight) Gamble (= *Eugenia ?montana* Wight), India, Tamil Nadu, Nilgiris, prope Gudalur, K A Pirozynski (318a), 22.II.1966, DAOM 145762 (holotypus), IMI 120229a (isotypus).

Colonies hypophyllous, black, densely velutinous, more or less circular in outline, up to 14 mm diam. larger by confluence. *Superficial mycelium* composed of pale to dark brown, smooth, branched, radiating hyphae 7·2–9 μm wide with cells up to 90 μm long and pore-like anastomoses between occasional contiguous hyphae. *Stomatopodia* lateral, scattered, concolorous with the hyphae, mostly sessile, sometimes shortly stalked, subglobose to ellipsoidal to obovoid, straight or bent, up to 29 μm long and 9–12 μm wide, 1-celled, sometimes producing one or two lateral hyphae. From the appressed surface each stomatopodium produces a hypha which penetrates a stomate: the hypha is brown to dark brown, usually swollen below the stomate itself and then branched within the substomatal chamber, gradually becoming hyaline and invading the leaf intercellularly at least as far as the palisade tissue. No haustoria were observed. Just above the stomatal aperture is an ellipsoidal cavity formed in the thickened outer wall of the epidermal layer. The invading hypha expands within this cavity. When colonies are removed from the leaf surface by the collodion technique this hyphal swelling may remain within the cavity or it may be torn away attached to the stomatopodium. In the latter circumstance the swelling appears as an additional subglobose to ellipsoidal cell attached to the appressed surface of the stomatopodium.

At the margin of a few of the larger colonies some diminutive colonies of the fungus up to 1 mm diam. have been seen, and a few bear occasional phialophores. Each of these colonies has arisen from a hypha which has reached the leaf surface from within the leaf tissues by growing through a stomate: at the leaf surface a swollen dark brown cell is produced which finally develops 3 or 4 hyphae that grow over the leaf surface. The central cell of such secondary colonies bears an ellipsoidal scar on the appressed surface. Such colonies are scanty in this species but are exceedingly abundant in some others.

Phialophores numerous and densely crowded, setiform, erect, arising laterally on superficial hyphae, 1–4(–5)-dichotomously branched and arborescent. They are thick-

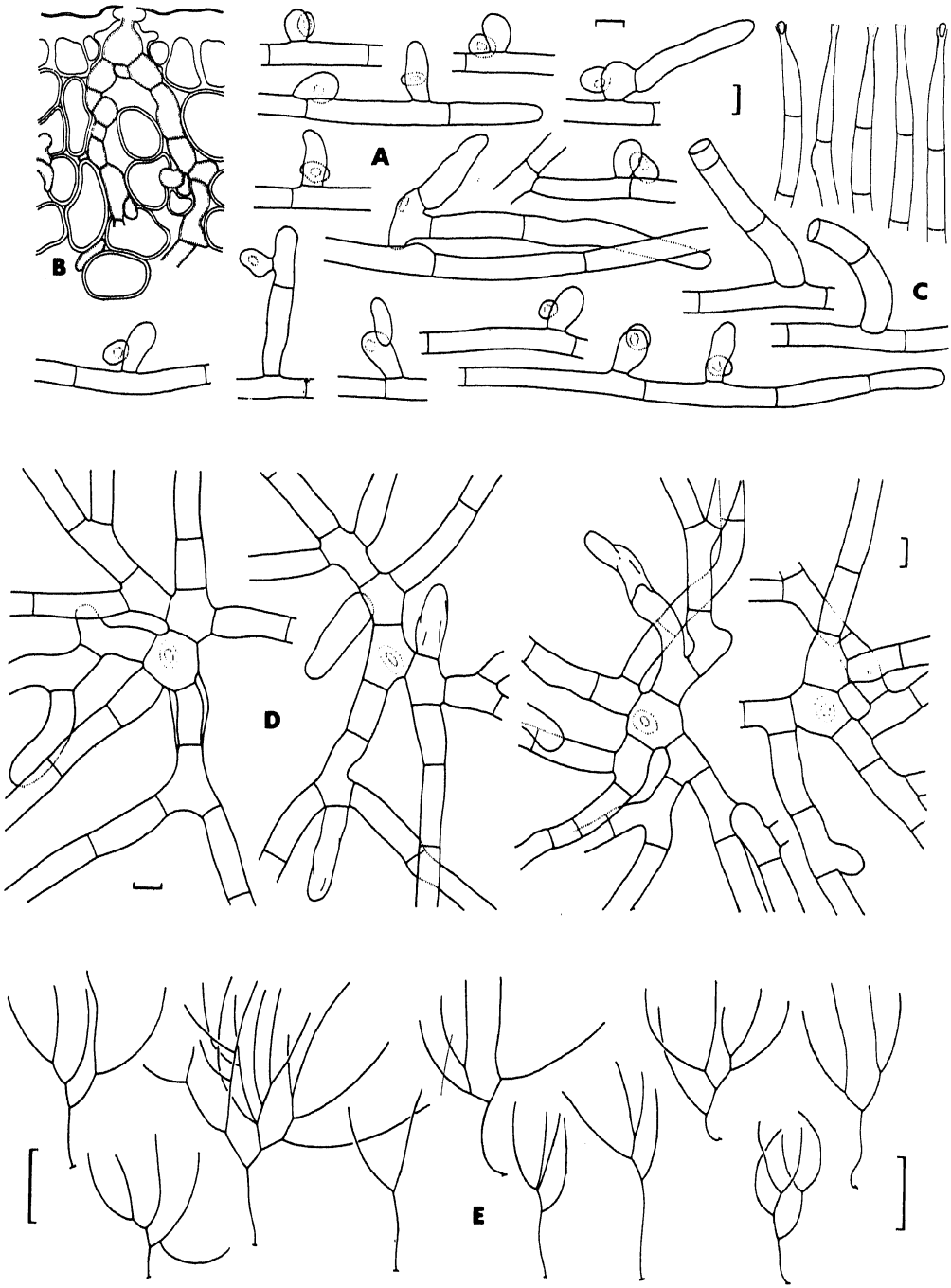


Figure 1. *Meliolina subramanianii.* **A.** superficial hyphae and stomatopodia. **B.** vertical section through leaf (longitudinally through stomate) showing intercellular hyphae, substomatal hyphae and torn hyphal cell below outer stomatal cavity. **C.** bases of two phialophores and five phialides with phialoconidia. **D.** central part of small secondary colonies with scar on the cell which has emerged through a stomate. **E.** phialophores showing 1–5 dichotomies. Semidiagramatic: phialophore thickness not shown. From the holotype. (Scale bars in A–D are 10 μm, in E, 100 μm.)

walled, very dark brown and 9–11 μm wide just above the base, tapering gradually and becoming paler to brown and thinner-walled toward the ends of the branches: they are up to 370 μm long with most ends terminating in a phialide. Phialides are narrowly obclavate to more or less subulate, 34–48 μm long, pale brown and 5·4–8 μm wide at the base, subhyaline toward the narrowed apex which is 2·3–2·7 μm wide below a funnel-shaped collarette. Phialoconidia (presumably spermatia) are cuneiform, hyaline, 4–5 μm long and 2·1–2·5 μm wide: they gather in a group at the end of the phialides.

Perithecia arise on the superficial mycelium and are at first obscured by the arborescent phialophores. At maturity they are globose up to 450 μm wide and 350 μm high, ostiolate, and they bear numerous unbranched, radiating, septate, thick-walled, dark brown setae up to 330 μm long, 7–9 μm wide at the base tapering to 4–7 μm wide at a bluntly rounded apex. The perithecium wall is 40–50 μm thick with an outer dark brown layer of thick-walled cells merging with an inner layer of thin-walled subhyaline and tangentially flattened cells. Asci clavate to narrowly obovoid, 160–170 × 50–55 μm, (4–)8-spored, relatively undifferentiated. Paraphyses conspicuous, unbranched or once branched toward the base, septate and barely constricted at the septa, hyaline 5–7·2(–9) μm wide at the base, tapering imperceptibly to 5–6·5 μm at the bluntly rounded apex.

Ascospores ellipsoidal to subcylindrical, subpapillate to papillate at each end, brown to dark brown, 3-septate, constricted at the septa, with a darkened and raised ring around the septal pores and a thin, darker band of wall partially obscuring the septa. At the apex and base the inner wall forms a conspicuous, circular, convex, dark brown, centrally thinner disc. The end cells show two transverse, circumferential, narrow, subhyaline bands, each representing a thinner zone in the ascospore wall, one next to each darkened disc and the other near the septum. Ascospores measure 50–70 × 18–23·4 μm.

On living leaves of *Syzygium ?montanum* (Wight)Gamble (= *Eugenia montana* Wight), India, Tamil Nadu, Nilgiris, near Gudalur, K A Pirozynski (318a), 22.II.1966, DAOM 145762 (holotype), IMI 120229a (isotype).

Meliolina subramanianii differs from other species of the genus in its papillate ascospores. '*Meliolina mollis* (Berk. & Br.) Höhnel *sensu* Höhnel (1910, 1919) (= *M. pulcherrima* (H & P Syd.) H & P Sydow 1914) differs in its longer, narrower ascospores with bluntly rounded ends and mostly 8 hyaline bands two to each cell.

It is with the greatest pleasure that we name this species to honour Prof. C V Subramanian whose friendship we have treasured for many years.

Wynaad is the site of another collection of *Meliolina*. The fungus was recorded as *Meliola cladotricha* Lév. (Sydow *et al* 1911) and subsequently listed under this name by Butler and Bisby (1931) and Vasudeva (1960). The same collection was listed by Mukerji and Juneja (1975) but *sub Meliolina arborescens* (H & P Syd.) H & P Syd. according to a re-identification by Kapoor (1967). The material (in Herb. HCIO and IMI) collected by W McRae on *Eugenia jambolana* Lam. (= *Syzygium cuminii* (L) Skeels) in Cottamunda on 22 Nov. 1909 has been examined by us and found to match *M. pulcherrima*. Hansford (1946) listed this collection under *M. mollis*.

Particulars of three other occurrences of *Meliolina* in India have been recorded in the literature:

(i) A specimen on leaves claimed to be *Syzygium jambolanum* (Lam.)DC from Poona was recorded as *Meliolina cladotricha* (Uppal *et al* 1935). Portion of this collection

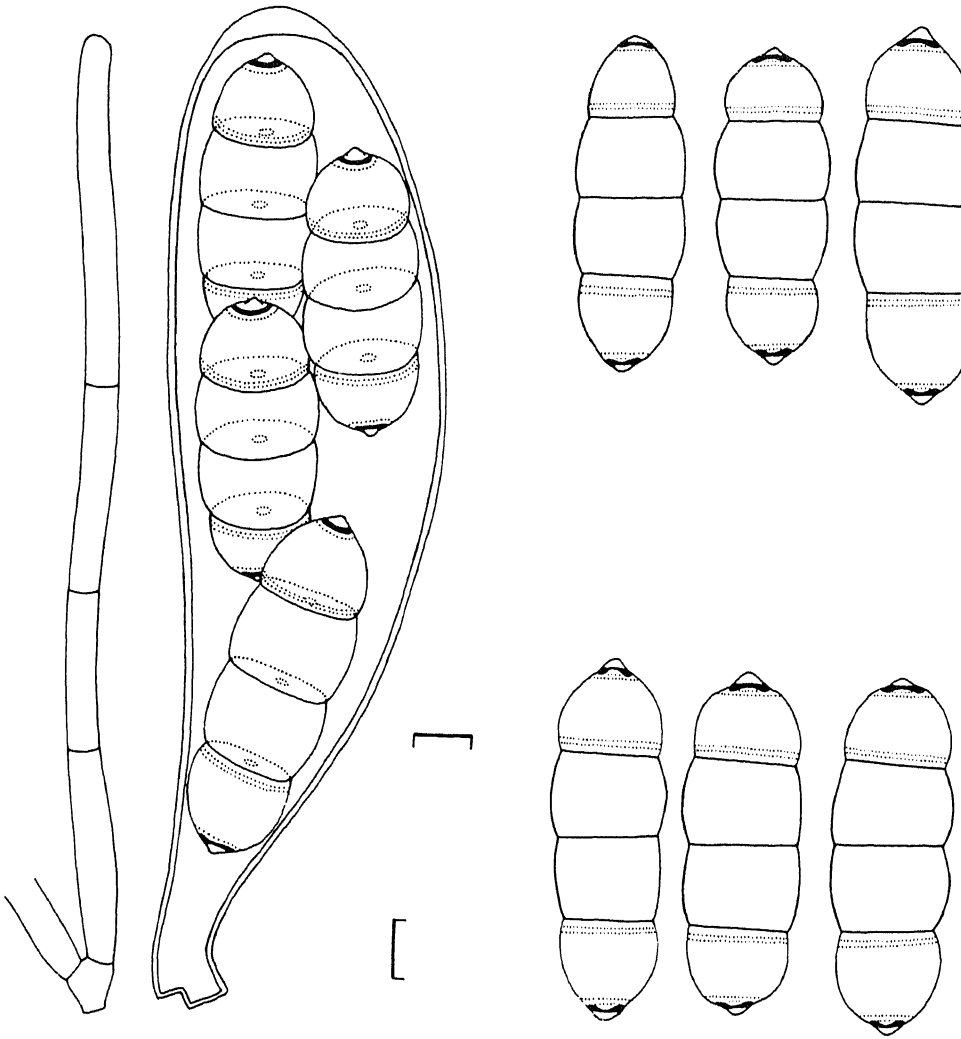


Figure 2. *Meliolina subramanianii.* A four-spored ascus, paraphysis and six loose ascospores. From the holotype. (Scale bars are 10 μm.)

(in Herb. F) has been annotated by Hansford to the effect that the mycelium found on leaves appears to be chaetothyriaceous. The host lacks laminar glands and is not, therefore, a *Syzygium.*

(ii) The specimen listed by Hansford (1946) as 'Hooker, s.n. on Myrtaceae. Khasia, India in Herb. Kew' is also sterile. The packet (in Herb. K) is illegibly labelled with what looks like 'Amure, Khasia' which could refer to the Kharia distinct of the Chota-Nagpur Plateau or, more likely, to the Khasi Hills of Assam.

(iii) *Meliolina pulcherrima* was listed by Mukerji and Janeja (1975) with reference to Ponappa (1967) who collected it on *Syzygium cuminii* in Murnad–Coorg, Karnataka. We have not seen this specimen.

Figure 3. *Meliolina subramanianii*. Young and mature ascospores. From the holotype (×730).

In addition, the following herbarium specimens (in Herb. IMI) from India bear species of *Meliolina*:

(i) Another collection from Coorg on *Syzygium cuminii*.
(ii) A University of Gorakhpur specimen on *S. tetragonum* Kurz [originating from the foothills of the Nepalese Himalayas?]
(iii) Three specimens collected in Burnihat, Assam: (a) on unidentified host, apparently *Syzygium*, (b) on *S. cuminii*, and (c) on "*Terminalia bellerica* Roxb". The latter entry calls for the verification of the identity of the host because this is the only record of the occurrence of *Meliolina* on Myrtales other than the Myrtaceae. The fragments of leaves comprising this specimen show numerous spherical laminar glands the presence of which is inconsistent with the host determination.

Both from the herbarium and the published records, *Meliolina* in India has a clearly disjunct distribution: the higher elevations in the Malabar section of the Western Ghats, and the foothills of the eastern Himalayas. The present-day pattern appears to have been imposed by the discontinuity of the combination of host and wet, tropical, montane habitat rather than that of the potential host alone. *Syzygium* is more widely distributed along Western Ghats from the latitude of Bombay to Cape Comorin, the Himalayan foothills of Sikkim and Bhutan, North Cahar, Khasi, Garo and Lushai Hills (in the Chittagong area) in Assam, Balaghat and Canar in the Satpura Range of Madhya Pradesh, Seshachalam Hills in Andhra Pradesh and Javadi Hills in North Arcot, Tamil Nadu. *Syzygium cuminii* is extensively cultivated in all but the driest parts of India.

The overwhelming majority of the Indian Myrtaceae is concentrated in the southern end of the peninsula. The southern ranges of the Western Ghats and Wynaad–Nilgiri especially is an important centre of endemism (Blasco 1971) and the home of the monotypic *Meteoromyrtus wynaadensis* Gamble a relative of *Syzygium* (Aubréville 1974), and of some members of *Syzygium* itself. *S. montanum* (Wight) Gamble, the probable host of *M. subramanianii*, is strictly endemic to dense forests of the Nilgiri Plateau at about 1800 m, being absent from similar ecosystems even of the neighbouring Palni (Blasco 1971).

The flora of peninsular India, both fossil and living, shares many elements with the floras of Africa and Australia (Axelrod and Raven 1978; Specht 1981). The *Syzygium*/*Meliolina* association provides a further example of this vicarious relationship of India to its former Gondwanalandian neighbours of Africa and Australia. In all three of these now discrete lands most of the original mesophytic floras have been eliminated, modified or fragmented and forced into more humid montane refugia. In all three the *Syzygium*/*Meliolina* associations are found in precisely such locations.

To students of Indian fungi, especially those following Subramanian's footsteps in the Wynaad–Nilgiri, to those puzzled by consistent similarities of the mycota of India, intertropical Africa and Australo-Papua/New Zealand, we offer a reminder: the mycological road from Ootacamund winds its way to Mysore through Gudalur, Brisbane and Entebbe.

References

Aubréville A 1974 Origines polytropiques des angiospermes tropicales (2^{e} partie); *Adansonia* **14** 145–198

Axelrod D I and Raven P H 1978 Late Cretaceous and Tertiary vegetation history of Africa. In *Biogeography and ecology of Southern Africa* (ed.) M J A Werger (The Hague: W. Junk) p. 77–133

Blasco F 1971 Montagnes du sud de l'Inde; *Trav. Sect. Sci. Tech. Inst. Fr. Pondichéry* **10** 1–436
Butler E J and Bisby G R 1931 The Fungi of India; *Sci. Monogr. Imp. Council Agric. Res.* **1** 1–237
Hansford C G 1946 Contributions towards the fungus flora of Uganda. VIII. New Records; *Proc. Linn. Soc. London* **157** 138–212
Höhnel F von 1910 Fragmente zur Mykologie (X. Mitteilung, Nr 468 bis 526); *Sitzungsber. Akad. Wiss. Wien, Math.–naturwiss. Kl.* Abt. 1 **119** 393–473
Höhnel F von 1919 Fragmente zur Mykologie (XXIII Mitteilung, Nr 1154 bis 1188); *Sitzungsber. Akad. Wiss. Wien, Math.–naturwiss. Kl.* Abt. 1 **128** 535–625
Kapoor J N 1967 Notes on Indian Meliolinae; *Indian Phytopathol.* **20** 151–160
Mukerji K G and Juneja R C 1975 *Fungi of India* (Delhi: Emkay)
Pirozynski K A 1974 *Meliolina mollis* and two hyperparasites in India; *Kavaka* **2** 33–41
Ponappa K M 1967 Some interesting fungi—I. Miscellaneous fungi; *Proc. Indian Acad. Sci.* (*Plant Sci.*) **66** 266–272
Specht R L 1981 Evolution of the Australian flora: some generalizations. In *Ecological biogeography of Australia* (ed.) A Keast (The Hague: W. Junk) **1** 783–805
Sydow H and Sydow P 1914 Diagnosen neuer philippinischer Pilze; *Ann. Mycol.* **12** 545–576
Sydow H, Sydow P and Butler E J 1911 Fungi Indiae Orientalis; *Ann. Mycol.* **9** 372–421
Uppal B N, Patel M K and Kamat M N 1935 The Fungi of Bombay; *Bull. Bombay Dept. Agric.* **176**.
Vasudeva R S 1960 The *fungi of India* (New Delhi: ICAR)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 355–361.
© Printed in India.

Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle*

JAN KOHLMEYER

Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, North Carolina 28557, USA

Abstract. A new marine Ascomycete, *Caryosporella rhizophorae* Kohlm. gen. et sp. nov., is described from dead wood of intertidal roots and branches of *Rhizophora mangle* growing on three small islands off the coast of Belize (Central America). The new genus is compared with *Caryospora*, and its placement in the Melanommatales and Massariaceae is discussed. The new species is considered to be an obligate marine fungus, but because of its taxonomic position it belongs to the group of secondary marine fungi that originated from terrestrial ancestors.

Keywords. Marine fungi; Ascomycetes; *Rhizophora;* mangrove; *C. rhizophorae.*

1. Introduction

In the course of studies of mangrove-inhabiting fungi (Kohlmeyer 1984), I encountered repeatedly an unidentifiable Ascomycete growing on decomposing roots of *Rhizophora mangle* in Belize (Central America). At first inspection the fungus appeared to be a terrestrial species with carbonaceous ascocarps that had survived the salt spray and occasional submergence in seawater in the mangal. However, close examination of 13 collections made on three different cays of the Atlantic barrier reef complex (Rützler and Macintyre 1982) showed that the Ascomycete was restricted to areas on the roots that bore marine organisms, such as shipworms, barnacles, bryozoa, *Spirorbis* sp., algae (*Bostrychia* sp.), and marine fungi. Therefore, it can undoubtedly be assigned to the obligate marine fungi (Kohlmeyer and Kohlmeyer 1979).

The new species can be included in the Melanommatales, Massariaceae, *sensu* Barr (1979a, b, 1983) and is close to the genus *Caryospora* de Not., however, not identical with it. Accordingly, a new genus and species are described in the following.

2. Description

Caryosporella Kohlm. gen. nov.

Genus Melanommatalium, Massariacearum. *Ascocarpiis* gregariis, grandibus, subglobosis, superficialibus, ostiolatis, periphysatis, papillatis, carbonaceis, nigris; *peridiis* crassis, tristratis; strato externo aureo, strato medio brunneo, strato intimo hyalino; stratis externis texturas epidermoideas formantibus; strato intimo texturam prismaticam formante; *pseudoparaphysibus* trabeculatis, ramificantibus anastomosantibusque super ascos, in matrice gelatinosa inclusis; *ascis* octosporis, cylindricis, pedunculatis, bitunicatis, pachydermis, apparatu apicale galeiforme, successive in textura ascogena in solo ventris ascocarpii maturescentibus; *ascosporis* ellipsoideis, unisep-

tatis, atro-brunneis, apicibus pallidis, obscure verruculosis; *typus generis: Caryosporella rhizophorae* Kohlm. sp. nov.

A genus of Melanommatales, Massariaceae with one obligately marine species. *Ascocarps* gregarious, large, subglobose, superficially seated on a thin black stroma, ostiolate, periphysate, papillate, carbonaceous, black. *Peridium* thick, three-layered, yellow on the outside, brown in the middle, hyaline near the centrum, the outer two layers forming a textura epidermoidea, the inner one a textura prismatica. *Pseudoparaphyses* trabeculate, thin, simple between the asci, branched and anastomosing above them, embedded in a gelatinous matrix. *Asci* eight-spored, cylindrical, pedunculate, bitunicate, thick-walled, with an apical cap-like apparatus, maturing successively on an ascogenous tissue at the bottom of the locule. *Ascospores* ellipsoidal, one-septate, dark brown with a light-colored tip, indistinctly verruculose. *Type species: Caryosporella rhizophorae* Kohlm. sp.nov. *Etymology:* From *Caryospora* de Not., a related genus, and the diminutive suffix *-ellus*, in reference to the relatively small ascospores in *Caryosporella.*

Caryosporella rhizophorae Kohlm. sp. nov.

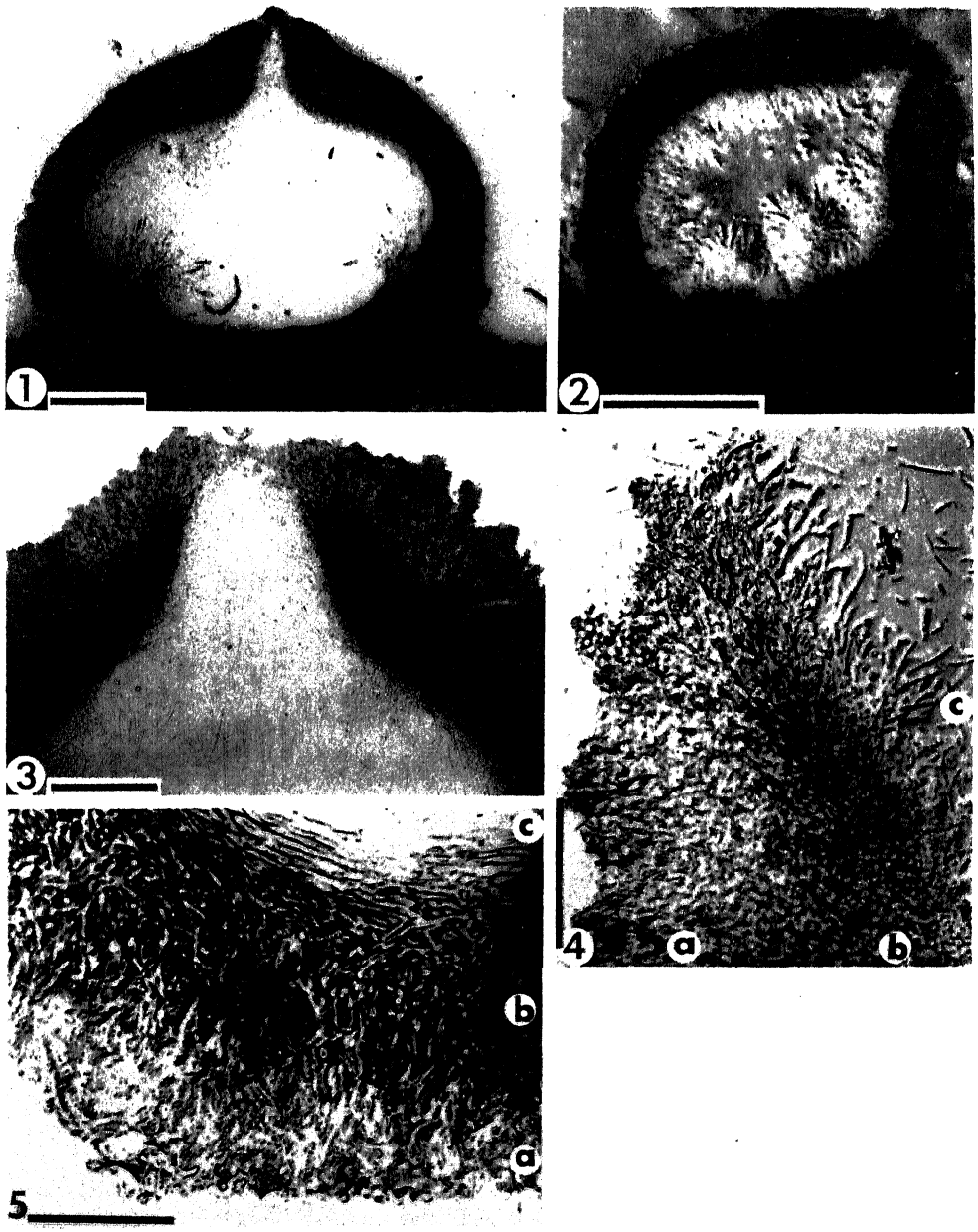
Ascocarpiis 750–900 μm altis, 830–1050 μm diam., gregariis, subglobosis, superficialibus supra stromatem tenue, ostiolatis, periphysatis, breve papillatis, carbonaceis, pachydermaticis, nigris, aspris; *papillis* 80–200 μm altis, 260–280 μm diam., canale ostioli 80–100 μm diam.; *peridiis* 90–160 μm crassis, tristratis; strato externo 30–50 μm crasso, aureo; strato medio 50–80 μm crasso, brunneo; strato intimo 15–25 μm crasso, dilute brunneo ad hyalino; stratis externis texturas epidermoideas formantibus; strato intimo texturam prismaticam formante; *pseudoparaphysibus* 2–3 μm diam., trabeculatis, ramificantibus anastomosantibusque super ascos, sensim septatis, in matrice gelatinosa inclusis; *ascis* 200–250 × 16–18 μm, octosporis, cylindricis, longipedunculatis, bitunicatis, pachydermis, apparatu apicale galeiforme, successive in textura ascogena in solo ventris ascocarpii maturescentibus; *ascosporis* (22–)24–30 × 10–12 μm, ellipsoideis, uniseptatis, ad septum leniter constrictis, atro-brunneis, apicibus pallidis, obscure verruculosis, pachydermaticis, monostichis imbricatisque in ascis. *Substratum:* lignum mortuum *Rhizophorae mangle* L. *Distributio:* Oceanus Atlanticus (Belize).

Holotypus: J.K. 4532a (NY); Isotypus: J. K. 4532b (IMS).

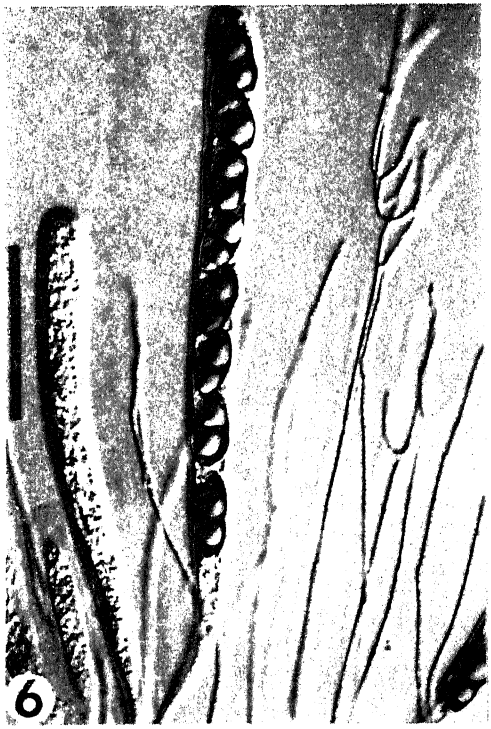
Caryosporella rhizophorae Kohlm. sp. nov.

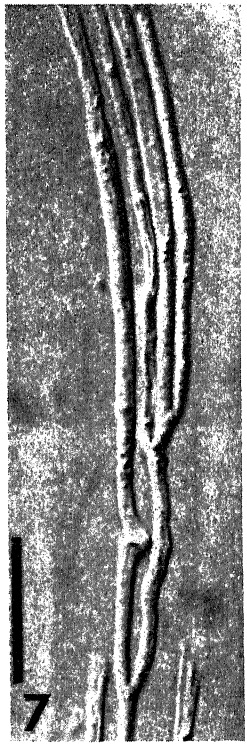
Ascocarps 750–900 μm high, 830–1050 μm in diameter, gregarious, often growing close together, subglobose, superficially seated on a thin black stroma that covers the wood surface, ostiolate, periphysate, short papillate, carbonaceous, thick-walled, appearing black, with a rough surface (figure 1). *Papilla* 80–200 μm high, 260–280 μm in diameter, ostiolar canal 80–100 μm in diameter (figures 3,4).

Peridium 90–160 μm thick, three-layered, outer layer 30–50 μm thick, golden yellow, middle layer 50–80 μm thick, deep brown, inner layer 15–25 μm thick, light brown to hyaline; the colour is caused by pigments encrusted on the walls; the outer two layers composed of thick-walled, irregular cells, forming a textura epidermoidea, the inner layer with elongate cells and large lumina, forming a textura prismatica, giving rise to pseudoparaphyses (figure 5); cells of the host are occasionally incorporated in the peridium. *Pseudoparaphyses* 2–3 μm in diameter, trabeculate, simple between the asci, branched and anastomosing above them, inconspicuously septate, embedded in a



Figures 1–5. *Caryosporella rhizophorae*. **1.** Ascocarp in longitudinal section (1 s. 10 μm), some immature asci on the bottom of the locule; scale = 200 μm **2.** Spermogonium? (1 s. 16 μm) attached to peridium of ascocarp; scale = 50 μm. **3.** Ostiole of immature ascocarp with periphyses and pseudoparaphyses (1 s. 4 μm); scale = 100 μm. **4.** Peridium, showing the **a.** outer and **b.** middle layer and **c.** periphyses; scale = 50 μm. **5.** Peridium with **a.** outer **b.** middle, and **c.** inner layer; scale = 50 μm. Figures 2 and 4 in Nomarski interference contrast, the others in brightfield; figures 3 and 4 from Herb. J.K. No. 4511, the others from J.K. No. 4590.

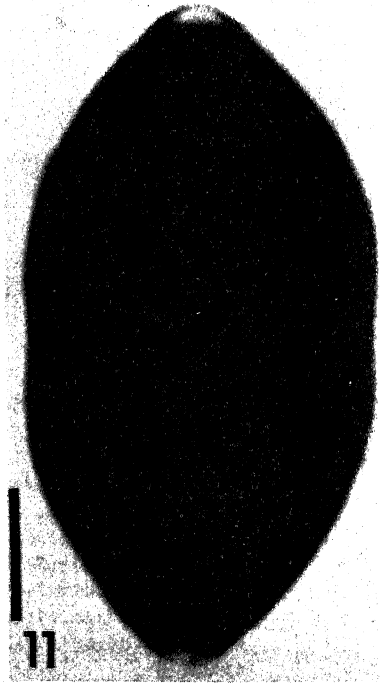

6

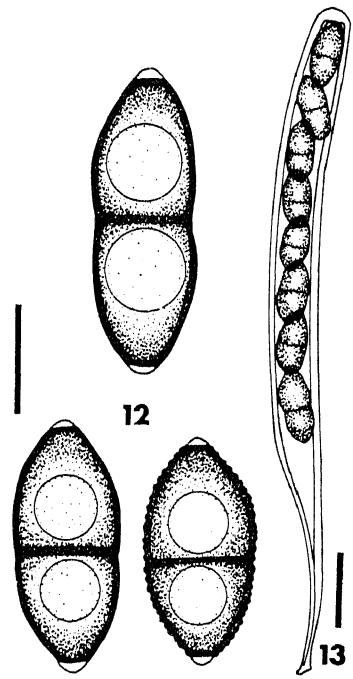

7

8

9

10


11



Figures 12 and 13. *Caryosporella rhizophorae.* **12.** Ascospores, one showing the surface sculpturings; scale = 10 μm. **13.** Ascus; scale = 25 μm; from Herb. J.K. No. 4590.

gelatinous matrix (figures 6,7). *Asci* 200–250 × 16–18 μm, eight-spored, cylindrical, long pedunculate, bitunicate, thick-walled (figures 6,13), with an apical cap-like, IKI-negative apparatus (figure 9), maturing successively on the ascogenous tissue at the bottom of the locule. *Ascospores* (22–)24–30 × 10–12 μm, ellipsoidal, one-septate, slightly constricted at the septum, dark brown, lighter coloured at the thickened apices (figures 10–12), indistinctly verruculose (visible under oil immersion; figure 8), thick-walled, overlapping monostichously arranged in the ascus (figure 6). Many ascocarps have one or more small black conidiomata (or spermogonia?) attached to the outside of the peridium (figure 2). Since the conidia are small, they are probably spermatia. *Substrate:* In the intertidal part of dead hard wood of roots and stems of *Rhizophora mangle,* next to woodborers, algae, and other marine life. *Range:* Known from three islands of the Atlantic barrier reef system in Belize, Central America. *Etymology:* Named after the host genus, *Rhizophora.*

Material examined: All collections on dead wood of intermittently submerged roots or branches of *Rhizophora mangle* in Belize; Twin Cays (16°50′N, 88°06′W), J.K. 4370,

---

Figures. 6–11. *Caryosporella rhizophorae.* **6.** Immature and mature ascus, trabeculate pseudoparaphyses; scale = 50 μm. **7.** Branching pseudoparaphysis, scale = 15 μm. **8.** Ascospores, showing verruculose surface; scale = 10 μm. **9.** Ascus tip with apical apparatus; scale = 15 μm. **10.** Immature ascospores, showing thick walls, except at the tips; scale = 10 μm. **11.** Mature ascospore with light-colored apices; scale = 5 μm. Figure 11 in brightfield, the others in Normarski interference contrast; all from Herb. J.K. No. 4590.

4371, 4375, 4507, 4511, 4523, 4531, 4532a (Holotype, NY), 4532b (Isotype, IMS), 4590, 4591, 4592, collected on 3 April 1983, 15, 17, 18 Mar. and 13 Nov. 1984; Wee Wee Cay (16°45′45″N, 88°09′W) J.K.4561, 25 Mar. 1984; Spruce Cay (16°43′N, 88°09′15″W), J.K. 4563, 25 Mar. 1984. Besides woodborers and other marine organisms, the specimens bear next to *Caryosporella rhizophorae* also the following marine Ascomycetes: *Dactylospora haliotrepha* (Kohlm. et Kohlm.) Hafellner, *Didymosphaeria rhizophorae* Kohlm. et Kohlm., *Halocyphina villosa* Kohlm. et Kohlm., *Hydronectria tethys* Kohlm. et Kohlm., *Leptosphaeria australiensis* (Cribb et Cribb) Hughes, *Lulworthia* sp. and other unidentified species.

Collection J.K. No. 4511 contains immature ascocarps in all stages of development. The fruiting bodies are superficial from the beginning, only some wooden cells may be occasionally enclosed in the peridium and taken up when the ascocarp expands. A small ascocarp of 420 μm in diameter was sectioned on the cryostat and showed a hyaline subglobose centrum, 180 μm in diameter, composed of mostly unbranched pseudoparaphyses. The peridium was already separated into a yellow outer and a brown middle layer, the ostiolar canal with some periphyses was visible, but the papilla had not formed as yet. At this stage of development there were no traces of ascogenous hyphae or asci. Another immature, but papillate (figure 3) ascocarp of 850 μm in diameter contained a dense ascogenous tissue at the bottom of the locule, between the peridium and the globule of trabeculate pseudoparaphyses. At a later stage, asci develop and grow up between the pseudoparaphyses.

3. Discussion

Caryosporella rhizophorae fits well into the Melanommatales (Barr 1979b, 1983) and Massariaceae (Barr 1979a). The only characters not agreeing with the latter family as it is defined by Barr (1979a), are the entirely superficial ascocarps and the medium-sized ascospores. Massariaceae are not recognized as a separate family by Eriksson (1981) who considers them to be a subfamily (Massarioideae) of Trypetheliaceae. *Caryosporella* could not be accommodated in Trypetheliaceae. The closest genus is *Caryospora* de Not. (Barr 1979a; Scheinpflug 1958), but ascocarps in *Caryosporella rhizophorae* are superficial from the beginning, the peridium is composed of 3 different layers of cells, the spores are thick-walled but not distoseptate and only of medium size.

Caryosporella rhizophorae is an obligate marine fungus that always occurs in association with other marine organisms, *viz*, algae, cirripeds, fungi and molluscs. Because of its taxonomic position in the Loculoascomycetes, I consider the genus *Caryosporella* to be derived from terrestrial ancestors and include it in the group of secondary marine fungi (Kohlmeyer and Kohlmeyer 1979). Typically, such secondary invaders of the marine environment are found in intertidal habitats, where extreme conditions, such as intermittent drying out and rehydration of the substrates, favor thick-walled ascocarps with periphyses, paraphyses or pseudoparaphyses that protect immature asci from complete dehydration. The bitunicate ascus is a characteristic example of a structure adapted to such habitats and not found in the primary marine fungi, such as representatives of Halosphaeriaceae (Kohlmeyer and Kohlmeyer 1979).

Caryosporella rhizophorae grows only on hard wood, usually on dead roots or branches that hang down into the water, but are dry or barely submersed at low tides. Apparently, ascocarps of *C. rhizophorae* take a long time to develop to maturity and

could not survive on bark or soft wood that is peeling off or becomes quickly riddled by woodborers.

Acknowledgements

Smithsonian Contribution No. 165 (Investigations of Marine Shallow-Water Ecosystems Program, Reef and Mangrove Study—Belize). A publication resulting partly from work done at the Smithsonian Institution's field station on Carrie Bow Cay, Belize (in part supported by the Exxon Corporation). Also partly supported by grant BSR-8407155 from the US National Science Foundation. I am grateful to K Rützler for making my work at Carrie Bow possible, and to the colleagues who kindly assisted me in the field (L D Coen, G Hagerman, G Hendler and H Paerl). Thanks are due to the staff of IMS (B Bebout, J B Garner, H Page, G Safrit and K Salesses) for technical, artistic and clerical help.

References

Barr M E 1979a On the Massariaceae in North America; *Mycotaxon* **9** 17–37

Barr M E 1979b A classification of Loculoascomycetes; *Mycologia* **71** 935–957

Barr M E 1983 The Ascomycete connection; *Mycologia* **75** 1–13

Eriksson O 1981 The families of bitunicate Ascomycetes; *Opera Bot.* **60** 1–220

Kohlmeyer J 1984 Tropical marine fungi; *Mar. Ecol.* (P.S.Z.N.I) **5** 329–378

Kohlmeyer J and Kohlmeyer E 1979 Marine Mycology (New York: Academic Press) p. 690

Rützler K and Macintyre I G (eds.) 1982 The Atlantic barrier reef ecosystem at Carrie Bow Cay, Belize, I Structure and communities; *Smithsonian Contrib. Mar. Sci.* **12** 1–539

Scheinpflug H 1958 Untersuchungen über die Gattung *Didymosphaeria* Fuckel und einige verwandte Gattungen; *Ber. Schweiz. Bot. Ges.* **68** 325–385

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 363–367.
© Printed in India.

Pseudascozonus, a new genus of Pezizales

J van BRUMMELEN
Rijksherbarium, P.O. Box 9514, 2300 RA Leiden, The Netherlands

Abstract. A new ascomycetous genus, *Pseudascozonus*, related to *Ascozonus* and *Thelebolus*, is proposed with *Pseudascozonus racemosporus*, sp. nov. as type species.

Keywords. Pezizales; Thelebolaceae; *Pseudascozonus*; fimicolous fungi; eugymnohymenial ascomata.

1. Introduction

During our studies of fimicolous ascomycetes, the development and growth of a remarkable fungus could be observed for a long period at different temperatures.

This fungus shows some outstanding features such as the complete absence of enclosing tissues, the somewhat dualistic opening mechanism of the ascus, and the unique way in which the ripe ascospores are united into a cluster. Apparently the fungus belongs to an undescribed species and the combination of characters necessitates the creation of a new genus within the Pezizales.

2. Descriptions

2.1 *Pseudascozonus Brummelen, gen. nov.*

Ascomata eugymnohymenialia, sessilia, minutissima. Hypothecium vix evolutum. Receptaculum (caro et excipulum) nullum. Asci late clavati vel obovoidei, apice rotundati, operculati vel bilabiati, ea re Ascozoni generis similes; parietibus iodo haud caerulescentibus. Ascosporae primum ellipsoideae, leaves, hyalinae. Fimicola.

Typus generis: *Pseudascozonus racemosporus* Brummelen.

Ascomata eugymnohymenial, sessile, very small. Hypothecium not clearly differentiated. Flesh and excipulum absent. Asci broad, with a rounded apex, operculate or bilabiate, resembling those of an *Ascozonus*; the wall not staining blue with iodine. Ascospores at first ellipsoid, smooth, hyaline. Fimicolous.

Etymology: From ψενδής, false, and the generic name *Ascozonus*.

Pseudascozonus racemosporus Brummelen, sp. nov.

Ascomata eugymnohymenalia, solitaria vel gregaria, superficialia, sessilia, 70–150 μm diam., 50–100 μm alta; initio subglobularia, denique magis semi-globularia vel lenticularia, incolorata; constans ex ascis confertim fasciculatis sine tegumentis. Receptaculum (caro et excipulum) nullum. Asci late clavati vel obovoidei, apice rotundati, dehiscentes operculo parvo rotundato (ca. 3 μm diam.) vel fissura irregulari aut bilabiata, 28–33 × 11–15 μm, 8-spori; pariete sine annulo incrassato, iodo haud caerulescenti. Ascosporae irregulariter dispositae in fasciculos, laxe conjunctae in

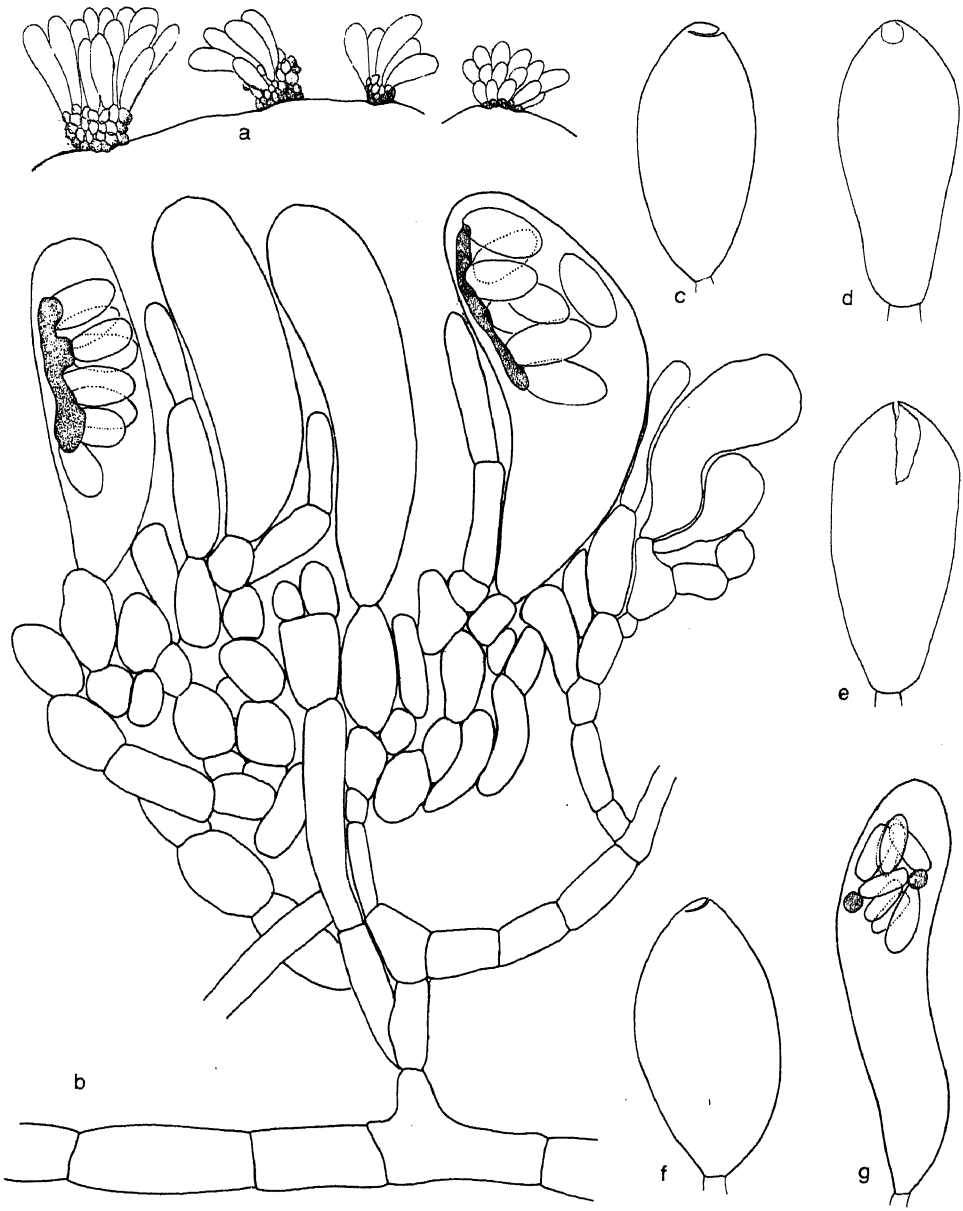


Figure 1. *Pseudascozonus racemosporus*. **a.** Fruit-bodies seen in side-view (× 125). **b.** Detail of a part of a fruit-body (× 1600). **c–g.** Asci (× 1000). (All from holotype.)

partem lateralem susbstantia amorpha luteola vel pallide brunneola, initio ellipsoideae vel cylindrico-ellipsoideae, deinde ovoideae vel clavatae, (6·6 –)8 – 10·2 (– 11·2) × (2) 2·5 – 3·6 (– 3·9) μm, laeves. Paraphyses nullae aut vix evolutae. In fimo cervi.

Typus: Gallia, *van Brummelen 7192* (holotypus, L).

Ascomata eugymnohymenial, solitary or in small groups, superficial, sessile, 70–150 μm diameter, 50–100 μm high; at first subglobular, then more semiglobular to

lenticular, colourless; consisting of a close fascicle of asci without any covering. Hymenium convex with the ripe asci protruding. Hypothecium not clearly differentiated, consisting only of a group of a few thin-walled cells, 6–15 × 2·3 – 3·5 μm. Flesh and excipulum absent. Asci broadly clavate to obovoid, with a thick base, rounded at the apex, opening by a small round operculum (about 3 μm across) or by an irregular of bilabiate split at the top, 28–33 × 11–15 μm, 8-spored; the wall without a ring-shaped thickening, not staining blue with iodine. Ascospores multi- or bi-seriate, irregularly arranged in a fascicle, loosely united at one side by small lumps of a yellowish or pale brownish amorphous substance, at first ellipsoid to cylindric-ellipsoid (length/width ratio 2·1–3), then ovoid to clavate, rarely allantoid (length/width ratio 3–4·4), often slightly curved at maturity, (6·6) 8 – 10·2 (– 11·2) × (2 –) 2·5 – 3·6 (– 3·9) μm, without oil drops or granules, smooth, without a secondary wall-layer. Paraphyses absent or represented by only a few scarcely differentiated, septate, irregularly cylindrical, sparsely branched, hyaline elements 2·5–3·5 μm thick.

Mycelium of colourless, septate hyphae 2–4·5 μm wide. Known only from dung of deer.

Etymology: From Latin, racemus, a bunch of grapes, and spora, a seed: with spores arranged into a bunch like grapes.

3. Material examined

France: *van Brummelen 7192*, on dung of deer (culture), Tourbière de Frasne, Frasne, dép. Doubs, 18.IV.1984 (type of *Pseudascozonus racemosporus* L).

4. Observations

This fungus with fruit-bodies, rarely over 120 μm wide, belongs to the smallest among the Pezizales. It is only because of the glistening white appearance that their fruit-bodies can be detected on the substratum with the help of a dissecting microscope.

The production of fruit-bodies occurs only at a rather late stage of the cycle of succession of fungi on a sample of dung. The fruit-bodies of *Pseudascozonus racemosporus* were not found before the third week after dung was laid in a moist chamber at room temperature (*ca.* 20°C). But even at much lower temperatures (*e.g.* 12°C) the regular production of fruit-bodies continued for many weeks after that. This slow development is probably also the reason why efforts to isolate this fungus in pure culture did not succeed.

In its way of growth this fungus resembles species of the genus *Ascodesmis* Tiegh. Both have eugymnohymenial ascomata without excipulum (*cf.* van Brummelen 1967, 1972). These are characteristic of a type of development in which the hymenium is fully exposed from the first until the maturation of the asci.

The asci in *Pseudascozonus* show a broad straight base and a top opening at maturity, either with a very small rather roughly delimitated operculum or by an irregular bilabiate split.

The ascospores are ellipsoid at first but elongate with age. At maturity their surface is smooth, apparently without secondary wall material. "De Bary-bubbles" could not be provoked in lactophenol or similar media.

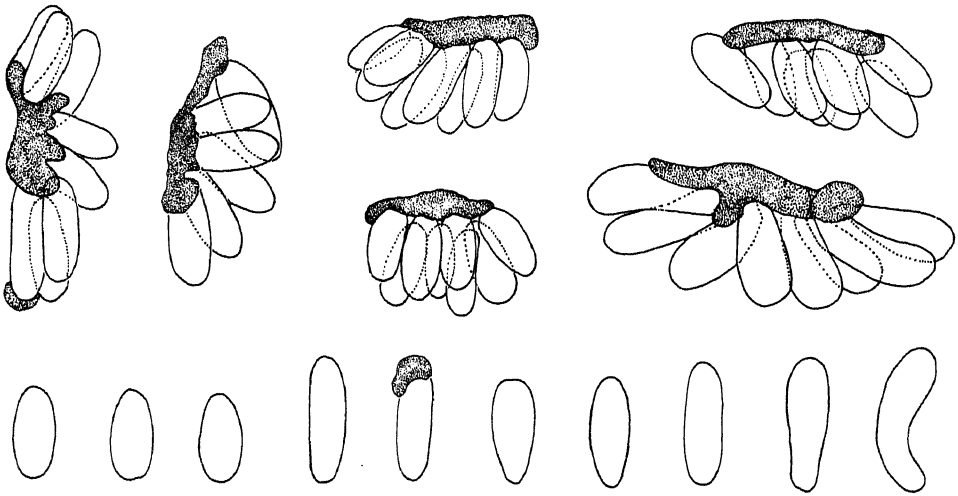


Figure 2. *Pseudascozonus racemosporus*, clustered and single ascospores (× 1600). (All from holotype).

Most or all of the eight spores in an ascus are rather loosely united at one side by several small or a single larger lump of a pale-coloured, amorphous substance. Occasionally one or a few free spores can also be observed in mature asci. After ejaculation of the contents of the asci in water the amorphous substance will gradually dissolve and the individual spores will become separate. This way of forming spore clusters is unique among the Pezizales. Perhaps something similar can be found in '*Ascophanus*' *coemansii* Boud. In this species mature ascospores are united by a hyaline mucilagenous reticulum dissolving in water soon after ejaculation.

5. Relationship

The general appearance of *Pseudascozonus* is much like that of *Ascodesmis*, but in the latter genus the fruit-bodies are larger and more gregarious, the asci have a very large, sharply delimited operculum at the top, and the ascospores are ornamented with brownish pigment. A position next to *Ascodesmis* in the Ascodesmidaceae is not tenable because of fundamental differences in the ascal and sporal structures (cf. van Brummelen 1981).

Very small eugymnohymenal ascomata without excipulum are also found in the doubtful genus *Zukalina* O.K. (Zukal 1887, as *Gymnodiscus*). Here the asci are multispored (probably 64 or more) with a cap-shaped top that is shot off at maturity. The spores are hyaline, fusiform and each one is surrounded by a broad mucilagenous envelope. *Zukalina* is certainly not related to our fungus.

If we compare the structure of the ascus and of the ascospores of this fungus with those of other genera of the Pezizales we see similarities with certain representatives of the family Thelebolaceae (Kimbrough 1966, 1972; Kimbrough and Korf 1967; van Brummelen 1974, 1978).

In this family different types of fruit-body ontogeny can be distinguished.

We find paragymnohymenial ascomata in *Coprotus* Korf (Kimbrough *et al* 1972),

while we even find eugymnohymenial ones in *Dennisiopsis* Subram. & Chandr. (Subramanian and Chandrashekara 1977). Both genera are characterized by asci with a large operculum and ascospores with "de Bary bubbles", much in contrast with our fungus. *Dennisiopsis octospora* Subram. & Chandr. may show a superficial resemblance, but the fruit-bodies are yellow and abundantly provided with long paraphyses.

Asci with a very small operculum and a bilabiate split are characteristic of the genus *Ascozonus* (Renny) E C Hansen. In this genus we find paragymnohymenial ascomata and asci with a prominent ring-shaped thickening in their wall and a subconical top (Renny 1871, 1873, 1874). The dehiscence of the ascus is initiated by the weakening of the wall at the margin of an apical disk, or a very small operculum. The disrupture of the ascus wall at this place is immediately followed by a bilabiate splitting of the wall in the ascus top above the ring (van Brummelen 1974). In *Pseudascozonus* such a ring-shaped thickening in the ascus wall is absent.

In the genus *Thelebolus* Tode, with cleistohymenial ascomata, we find somewhat similar asci among the species with a relatively low number of spores per ascus (van Brummelen 1978). Especially in species with 8-spored asci a roughly delimited operculum is found, while opening by an irregular tear is also possible.

Irrespective of the great differences in ascocarp ontogeny our fungus shows in its ascus structure undeniable affinities with *Ascozonus* as well as with certain species of *Thelebolus*.

References

Boudier J L E 1869 Mémoire sur les Ascobolacés; *Ann. Sci. Nat.* (*Bot.*) Sér. V **10** 191–268

Brummelen J van 1967 A world-monograph of the genera Ascobolus and Saccobolus (Ascomycetes, Pezizales); *Persoonia* (Suppl.) **1** 1–260

Brummelen J van 1972 Ascocarp ontogeny and a natural classification of the Ascobolaceae; *Persoonia* **6** 389–394

Brummelen J van 1974 Light and electron microscopic studies of the ascus top in *Ascozonus woolhopensis*; *Persoonia* **8** 23–32

Brummelen J van 1978 The operculate ascus and allied forms; *Persoonia* **10** 113–128

Brummelen J van 1981 The genus *Ascodesmis* (Pezizales, Ascomycetes); *Persoonia* **11** 333–358

Kimbrough J W 1966 Studies in the Pseudoascobolaceae; *Can. J. Bot.* **44** 685–704

Kimbrough J W 1972 Ascal structure, ascocarp ontogeny, and a natural classification of the Thelebolaceae; *Persoonia* **6** 395–404

Kimbrough J W and Korf R P 1967 A synopsis of the genera and species of the tribe Theleboleae (= Pseudoascoboleae); *Am. J. Bot.* **54** 9–23

Kimbrough J W, Luck-Allen E R and Cain R F 1972 North American species of *Coprotus* (Thelebolaceae: Pezizales); *Can. J. Bot.* **50** 957–971

Renny J 1871 A description of some species of the genus *Ascobolus* new to England; *Trans. Woolhope Nat. Field Club* **1871** 45–48

Renny J 1873 New species of the genus *Ascobolus*; *Trans. Woolhope Nat. Field Club* **1872–3** 127–131

Renny J 1874 New species of the genus *Ascobolus*; *J. Bot. Lond.* **12** 353–357

Subramanian C V and Chandrashekara K V 1977 *Dennisiopsis*, a new genus of Discomycetes; *Kew Bull.* **31** 639–644

Zukal H 1887 Ueber einige neue Ascomyceten; *Verh. k. k. zool.-bot. Ges. Wien* **37** 39–46

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 369–380.
© Printed in India.

The species concept in Hymenochaetaceae (Fungi, Hymenomycetes)

E PARMASTO

Institute of Zoology and Botany, 202400 Tartu, Estonian SSR, USSR

Abstract. Species of the Hymenochaetaceae (Fungi, Hymenomycetes) are presumably homothallic and consequently of only uniparental reproduction. The number of species described is high (more than 100 in *Hymenochaete*, several hundreds in *Phellinus*, etc.) and their classification may be successful only when a clear species concept is consistently used. Type studies may give some help mainly for nomenclature; for taxonomy the study of numerous specimens of each species and a statistical approach is needed.

The biological species concept is inapplicable to uniparental fungi; its indirect application mentioned by some evolutionists is unrealizable. The recognition of sibling microspecies on the basis of the selection species concept is the proper foundation for the taxonomy of the Hymenochaetaceae.

By way of examples the taxonomy of several pairs of closely related Hymenochaetaceae species is discussed (*H. mougeotii* and *H. murashkinskyi*; *H. rheicolor* and *H. attenuata*; *H. semistupposa* and *H. yasudai*; *Phellinus laevigatus* and *Ph. orienticus*; *Ph. pini* and *Ph. chrysoloma*; *Phylloporia ribis* and *Ph. ephedrae*). A new combination *Phylloporia ephedrae* (Voronich.) Parm. is proposed.

Keywords. Biological species concept; Hymenochaetaceae; microspecies; selection species concept; sibling species; type studies in fungi.

1. Introduction

Many biologists have asserted in the past decade that only less than a half of the species of organisms (including fungi) occurring in the tropics and the subtropics have been described until now. The vanishing of tropical forests and other natural landscapes together with many species is taking place rapidly, and that is why the promotion of taxonomic studies is an urgent need in all groups of organisms. The school of mycologists at Madras University headed by Prof. C V Subramanian has done an enormous amount of work in describing the diversity of fungi and their up-to-date taxonomy. This paper was compiled five years after its author was happy to see the rich fungal flora of South India and undertake a one-month visit to the Madras Centre for Advanced Study in Botany; nevertheless, the influence of these days on the formation of the ideas presented in this article has been influential.

The number of the undescribed species of fungi of the tropical countries must be very high; at the same time there are numerous species including wood-rotting fungi which have been described within the past one-and-a-half centuries which have never been re-described at the present-day level of science. One of the examples of such a group is the genus *Hymenochaete*. It is a clearly delimited and easily recognizable genus with more than 100 species described, but nobody after Massee (1891) and Cunningham (1963) has attempted to study the tropical species of this genus. Most of the species are described on the basis of only one or few collections without indicating reliable data on the microscopic structure. Most of the herbarium specimens including many types are sterile (*i.e.* without spores), and so is the possibility of comparing them rather

restricted. Lack of spores in many type specimens of the Aphyllophorales is a common fact (*cf* Parmasto 1978); many collections were made in a season when these fungi did not sporulate. Other specimens lost their spores when dried too slowly; thin-walled spores became decomposed under the action of bacteria. That is why it is urgently recommended to take a spore print from the specimens collected within a few hours (if necessary, after moistening the specimen), to keep the spore print in dry conditions and it is desirable to measure 30 or 50 spores of each sample as soon as possible.

Even after having good collections of the Hymenochaetaceae it is difficult to distinguish the species. The intraspecific variability seems to be enormous, the differences between the species are small and vague, the "concepts" of species used by different mycologists are very different. The genera *Phellinus*, to some degree also *Hydnochaete* and *Inonotus* have been monographically studied by Ryvarden and Johansen (1980), Pegler (1964) and Ryvarden (1982), respectively. The genus *Hymenochaete* remained unstudied; nobody has so far attempted to study the types of its species. Type studies, so modern nowadays, will render us no help until a preliminary picture of the species of the genus under study has been achieved.

The difficulties in the delimitation of the species in the Hymenochaetaceae are typical of the studies of many (especially tropical) Hymenomycetes. A taxonomic study may be quite fruitful only when there is full clarity about which species concept and which species criteria will be used. Many fungal taxonomists are convinced, that "a species is what is considered to be so by a well-informed taxonomist" (see Beaudry 1960). Many taxonomists believe that species may be split or lumped together according to the will of a scientist, *i.e.* that species do not really exist in nature. The collection of papers by Clémençon (1977) shows how widespread is the tendency to regard a fungal species as something very different from the species of other organisms, which needs completely new concepts, definitions and terms. Not denying *some* specific features of species in fungi (as well as in other major groups of organisms), the author of this paper wholly agrees with the opinion of Petersen (1977). "We must welcome the best methodology and terminology from other disciplines and biological kingdoms . . ."

2. Sibling species in fungi

In all groups of organisms there are pairs or clusters of closely related species, morphologically very similar but reproductively isolated. They usually differ distinctly from each other ecologically, many of them have different distribution areas. The term *sibling species* was introduced for such species by Mayr (1942) and is widely used in zoology and to a lesser extent also in plant taxonomy nowadays (see Mayr 1948; Grant 1981; White 1978, etc).

In mycology such species have been named *microspecies* (see Watling 1977; Kemp 1977, and obviously several others); however, this term is used in several meanings and it is better to reserve it for a different phenomenon (see below). Bas (1977) has used the term "*small species*" (in parentheses!) which is really a translation of the same term into English. Romagnesi (1977) has proposed the term *pseudovariété*. The term *sibling species* is used in the taxonomy of the Aphyllophorales obviously only by Petersen (1977). Burnett (1983) wrote that sibling species may be widespread in fungi; however, he mainly stressed the possible origin of sibling species by sympatric speciation, and he almost put inter-sterile groups in fungi on a par with sibling species.

We are convinced that sibling species is the most suitable term for the species distinguishable in many Hymenomycetes only with careful studies. In extreme cases sibling species may be differentiated only by means of biometrical techniques and with incompatibility tests; such cases are common, for example, when studying the genus *Peniophora* (Corticiaceae).

We wholly agree with Grant (1957) that "the distinction between sibling species and normal [*sic*! – E P] species is relative. Most sibling species when studied long enough and hard enough are separable morphologically". In the genus *Peniophora* we may see the species identifiable with the naked eye (as *P. laeta* (Fr.) Donk) and a pair of species without any differences except incompatibility (*P. quercina* (Pers.: Fr.) Cke. and *P. simulans* Reid). In the genus *Phellinus* there are species which are easily recognizable (*Ph. tremulae* (Bond.) Bond. et Boris.) and a cluster of very closely related species united earlier under the name *Ph. igniarius* (L.: Fr.) Quél.; however, the sibling species of this cluster are difficult to describe scientifically, but any experienced forester is able to distinguish them at a glance.

Many taxonomists feel no enthusiasm about the idea of the reality of sibling species. Several taxonomists have announced what a species *must* be: " . . . a precept in taxonomy should be, in our opinion, that valid species be really identifiable" (Snyder and Hansen 1954); "Morphological differences [of a species] from related species must be clearly expressed in the habitus of a plant, and visible even by superficial investigation with the naked eye . . ." (Bochancev and Lipshic 1955).

The desire to have species created in such a way that they would be easily distinguishable by *Homo sapiens*, a poor creature having weak eyes and blunt other senses, is quite understandable but unfortunately quite unrealistic. These views reflect nominalist principles in taxonomy characteristic of the biology of the eighteenth century.

The groups of sibling species have been treated as a single *taxonomic species* for purposes of routine identification (Grant 1971). Another possibility proposed by several biologists is to use Mayr's (1942) term *superspecies*, which is a "monophyletic group of distinct species that are allopatric in distribution" (Stanley 1979). As an arbitrary term *superspecies*, *collective species* or *macrospecies* may have some use in mycology, for example, to help amateur mycologists to orientate themselves in the diversity of the fungi. For applied mycology and phytopathology these terms and the lumping together of sibling species must be avoided: closely related sibling species are usually quite different from each other in their pathogenicity, specialization, etc. Logically these terms are nonsensical: two (or several) species cannot constitute one species.

3. Microspecies in fungi

The biological species concept is nowadays widely recognized as a concept in agreement with our knowledge of microevolution. It is considered to be not very operational in practice (see Sokal and Crovello 1970) but is nevertheless successfully applied, for example, in the taxonomy of several groups of Hymenomycetes. It is the theoretical background to the use of the incompatibility test in taxonomy at species level (see Boidin 1977; Boidin and Lanquetin 1984).

As worded by Mayr (1969), "Species are groups of interbreeding natural populations that are reproductively isolated from other such groups". Such a concept is

obviously not applicable to the organisms with uniparental reproduction (asexual species, unisexual species, apomictic groups, but also primarily homothallic fungi); this has been stated by several biologists, *e.g.* Heywood (1963) concerning persistent inbreeders. However, there are two contrary tendencies to draw conclusions from these facts.

The orthodox supporters of the biological species concept are convinced that only those groups of organisms (populations) that correspond to the concept may be called species. Already Turrill (1939) declared that apomicts are "neither varieties nor species". Grant (1957) states that "when sexuality is absent, the biological species concept breaks down"; "the biological species concept itself denies the existence of definite species units in certain plant groups". Some authors (Dobzhansky; see Wiley 1981) call the asexual species *pseudospecies*. Timofeeff-Ressovsky *et al* (1977) declared: in agamic forms, in forms with obligately vegetative reproduction, parthenogenetic and obligately autogamic forms the term species (in its exact meaning) is inapplicable.

In turn, some biologists have expressed vague hints at the possibility of applying the biological species concept also to non-interbreeding groups of organisms. Mayr (1957) himself wrote about the possibility of applying the biological species concept even to situations where it cannot be applied directly (for one reason or another). Löve (1964) stated that "the biological species concept is the only such definition that is universal and equally applicable to all biota . . . and, by proper inference from characters other than reproduction, even for taxa sexuality of which has been replaced by apomixis . . ." Stafleu (1969) advised: "Even if one cannot apply this biological species concept in particular cases . . ., it provides a background for the biologist in the evaluation of his more "old-fashioned" characteristics and combination of formal affinities."

But there are biologists who consider the uniparental groups to be real species. DuRietz (1930) found that to each apomict should be given the status of species; this has been widely followed by many taxonomists working on *Hieracium*, *Taraxacum* apomictic genera. However, Camp and Gilly (1943) said that this has led us into a "ludicrous state". Wiley (1981) recommended that in asexual species "where two clones can be adequately diagnosed, the naming of species is reasonable. Where two clones cannot be diagnosed but are differentiated only on a statistical level, the naming of species may be arbitrary and I would not advocate it". For Simpson (1961) uniparental and biparental populations "both form species and, by appropriate definition, the same kind of species".

For Wiley (1981) "agamospermic and parthenogenetic populations represent independent evolutionary lineages and thus are evolutionary species"; he states that the force promoting maintenance of species identity in asexual clades is evolutionary stasis (homeostasis). Ehrlich and Raven (1969) expressed the same idea more exactly: organisms with asexual reproduction "tend to occur as phenetic species—presumably groups of individuals being kept similar by their continued existence under similar selective regimes . . ." A species concept suitable for both biparental and uniparental populations has been proposed by Slobodchikoff (1976): this is the *selection species concept*. "A species is a system of genetically similar individuals and populations maintained as a cohesive unit by a set of selection pressures that balance the disruptive forces imposed by environmental factors, mutation, or genetic recombination"

This species concept is applicable to all uniparental fungi; unlike the taxonomical or

morphological species concepts, it explains why species occur in these organisms without (or with restricted) gene flow in populations.

As was demonstrated by Boidin (1958), all three species of *Hymenochaete* studied by him (*H. mougeotii* (Fr.) Cke., *H. rubiginosa* (Dicks.: Fr.) Lév. and *H. tabacina* (Sow.: Fr.) Lév.) are homothallic: their 1-nucleate basidiospores produce mycelia with 2 or many nuclei in the cells, and the monosporous mycelia do not differ from the mycelia of polysporous cultures. Most of the species of the Hymenochaetaceae studied have mycelia which are holocoenocytic, which is a sign of presumed homothallism (Boidin 1971). All the species of the Hymenochaetaceae have hyphal septa without clamps. The incompatibility test between the non-sib monosporous cultures of *Phellinus alni* (Bond.) Parm. demonstrated their antagonism, *i.e.* the same reaction as between the polysporous cultures; the same was observed also in *Ph. chrysoloma* (Fr.) Donk (Aguraiuja, unpublished data). According to this evidence, we may conclude that primary (?) homothallism is common, or very widespread in the Hymenochaetaceae.

The presumed homothallism of the Hymenochaetaceae species is the clue to the difficulties we have in the species discrimination of these fungi. The species are actually *microspecies* in the sense used by many botanists and defined by Grant (1981) as "populations in predominantly uniparental plant groups which are uniform themselves and are slightly differentiated morphologically from one another".

The microspecies are relatively well studied in higher plants, where apomictic species are numerous in several families. In these plants apomixis seems to be never absolute and there are many facultative apomicts. The age of apomictic species is comparable with many sexual species in some groups of *Alchemilla* (Walters 1972), 2–4 million years in some *Taraxacum* species, but a great number of those have descended only in the last glacial period in Europe (Doll 1982). In many cases the origin of apomictic species is connected with hybridization and polyploidy. The degree of difference between closely related taxa is different, and it is sometimes difficult to say, if a slight but statistically significant difference is a sufficient basis for taxonomic separation (Walters 1972). Many of the apomictic species have small distribution areas. Nevertheless, some asexual species are "as abundant, genetically variable, and geographically widespread as similar sexual species" (Stanley 1979).

Uniparental taxa in the Hymenochaetaceae are not quite similar to the apomicts or "asexual" species in plants. Polyploidy and hybridization are not recorded in these fungi. Many microspecies of the Hymenochaetaceae are widespread (*viz. Hymenochaete tabacina* (Sow.: Fr.) Lév., *H. rubiginosa* (Dicks.: Fr.) Lév., *Phellinus gilvus* (Schw.) Pat., *Ph. senex* (Nees et Mont.) Imaz., *Ph. tremulae* (Bond.) Bond. et Boris., *Inonotus dryadeus* (Pers.: Fr.) Murr., *I. radiatus* (Sow.: Fr.) P. Karst. and many others). Some species may be tens of millions of years old (*Phellinus laevigatus*). There is a number of closely related but easily distinguishable species in *Phellinus* and *Inonotus*.

The term *sibling species* has been used mainly in context with biological species. Would it be appropriate to combine the terms *sibling species* and *microspecies* for the taxa dealt with in this paper? Mayr (1963) wrote that "whether or not to list . . . clones as sibling species depend on the criteria adopted for 'species' in asexual organisms". DeBach (1969), who studied parasitic Hymenoptera (Insecta), proposed to divide biological species as well as uniparental species into two categories—morphological species and sibling species. Morphological species are taxa which have "discernable morphological differences with other species". This division proposed by DeBach

appears to be practical and is accepted here. However, in the examples of microspecies in the Hymenochaetaceae presented at the end of this paper the morphological microspecies are omitted; they raise much less problems in practical taxonomy than the sibling microspecies.

4. The definition of species and the standard of species in the Hymenochaetaceae

A species concept and a definition of species is not the same although the terms are sometimes confused (*e.g.* Löve in Mayr 1957; several debaters of the Herbette symposium on the species concept in the Hymenomycetes, see Clémençon 1977). One step further towards practical taxonomy is the *practical standard of a species*, *i.e.* the pattern of how to distinguish a species in a given group of organisms; it includes a complex of criteria useful for the taxonomy of the taxa dealt with (Zavadskij 1968). When a mycologist has written about a "small species concept" or about splitters and lumpers, he usually has in mind the practical standard used in the group of fungi under study. The same may partly be said also about the title of this paper.

Proceeding from the selectional species concept, four main groups of criteria may be used for species discrimination in the Hymenochaetaceae: morphological, biochemical, geographical and ecological criteria.

The use of morphological differences in the first place is the inevitable credit to be given to the morphological species concept. But we know that in the majority of cases "speciation has been accompanied or followed by morphological differentiation . . ." (Beaudry 1960).

One of the crucial questions when applying the species standard is the size and constancy of morphological (including micromorphological) differences. It seems that many authors of species definitions have overemphasized the meaning of constancy. Let us give some examples.

In the definition of species given by Huxley (1942): a species is distinguished from related species "by constant morphological differences". Bresinsky (in Bigelow 1977): "The main thing is that we have a hiatus, and that this hiatus is not overbridged by any kind of continuous variation . . ." Thiers (in Clémençon 1977): "A species is a population of individuals which possess a group of constant, reproducible characters in common . . ."

Actually, all the quantitative characters and most of the qualitative characters are variable in all species, and in many cases the closely related species have a partly overlapping variability. It is clearly seen in such very important characters as spore size (see Parmasto and Parmasto 1985). It is usually hopeless to search for a quite distinct, constant hiatus between related species. What is possible for us is to study the variability of characters using statistical methods and to evaluate the significance of the difference between the taxa under study.

This is a *desirable* approach which is rarely realizable due to the small number of specimens available to us. A large number of specimens (including types) of *Hymenochaete* and other Hymenochaetaceae in all herbaria are sterile, do not have any characteristic specific features, or seem to be intermediate between some species. Many of these specimens will never be identified with well-known species, and the best thing to do with such type specimens is to designate the species names based on these types as *nomina dubia*.

Biochemical criteria are rarely used in the study of the Hymenochaetaceae. The colour of the basidiomes is frequently used in the taxonomy of these fungi; the differences in the pigments which are responsible for colour may be studied using several analytical methods (see Parmasto and Parmasto 1979). There are a large number of methods to compare the biochemical differences between the cultures, the study of isoenzymes being the most promising. However, biochemical and physiological data used for comparison are usually obtained from the study of only one culture or a few cultures; but these data are not less variable between the specimens (cultures) than the micromorphological data.

Geographical criteria are usable due to the allopatric speciation being obviously as common in the Hymenochaetaceae as it is common in most other organisms. Most of the species of the Hymenochaetaceae are not so widely distributed as it is sometimes asserted due to the deficient exploration of the world fungal flora. Seemingly sympatric related species have usually descended by allopatric speciation, sympatry being in them a secondary phenomenon. All the well-studied species have characteristic areas of geographical distribution.

Ecological criteria are the most useful in the Hymenochaetaceae thanks to the obvious specialization of most species. Usually one species of *Phellinus* (or *Inonotus*) is growing on the wood of the species of one genus (or subgenus) of trees or shrubs. Unfortunately, many tropical and subtropical species of *Hymenochaete* have been collected without any data about their substrata, and that is one of the reasons why species delimitation is difficult in this genus. The same may be said of the tropical species of *Phellinus*.

5. Examples of the application of the concept of sibling microspecies in the Hymenochaetaceae

5.1 *Phellinus laevigatus* (Fr.) Bourd. et Galz. and *Ph. orienticus* Parm.

Ph. laevigatus s. l. is found everywhere in the forests of the temperate zone of the Northern Hemisphere; in North America it was for a long time considered to be a variety of *Ph. igniarius* (L.: Fr.) Quél.

Biometrical studies of the herbarium specimens and spore prints showed that there are two or three microspecies in this group of fungi. *Ph. laevigatus* s. str. has spores 4·3–5·5 × 3·6–4·1 μm (this is the observed range of the mean spore size of the specimens); setae are 15·7–22·3 × 5·3–6·8 μm (observed range of the specimen means). The species has spread from W. Europe to Central Siberia on wood of *Betula*.

Ph. orienticus has spores 2·9–4·1 × 2·4–3·1 μm and setae 14·1–17·9 × 4·7–5·9 μm. The species has spread from Central Siberia to the Soviet Far East and Japan on wood of *Betula*.

Ph. betulinus (Murr.) is widespread in North America; it may be an intermediate species, which, however, needs to be studied.

Judged by the distribution data and the history of the continents of the Northern Hemisphere, these three microspecies have descended from an ancient ring species which spread from East Asia eastwards in the early Eocene. Allopatric speciation took place after the separation of Europe from North America in the Eocene and after the separation of North America from Asia in the Pliocene. The boundary line between

Ph. laevigatus and *Ph. orienticus* in Central Siberia is clear and lies along the ancient Central-Siberian (Obik) Sea.

The difference between *Ph. laevigatus* and *Ph. orienticus* is statistically quite significant; nevertheless, this is an extreme case of the hardly noticeable difference between the microspecies. The taxa might be called *subspecies* of one species, but this term (category) is closely connected with the biological species concept which is inapplicable to uniparental organisms.

5.2 *Phellinus senex* (Nees et Mont.) Imaz. and *Ph. torulosus* (Pers.) Bourd. et Galz.

Ph. senex is a species of pantropical distribution; *Ph. torulosus* is distributed mainly in South Europe, in the Caucasus and south of the Caspian Sea, but is also found in North and South America. They are macroscopically so different that nobody has had any difficulties with their identification.

But there are some collections from North India which are externally intermediate between the two species; and some from the Crimea (USSR) where the basidiomes typical of both the species were found intermingled.

After this was ascertained, the author of this paper studied the specimens of both the species biometrically. *Ph. senex* has spores 5·1–5·6 × 3·7–4·4 μm and setae 25–37·9 × 6·2–8·5 μm (observed range of specimen means); *Ph. torulosus* has spores 5·1–5·8 × 3·6–4·2 μm and setae 24·4–38·5 × 6–8 μm. These differences are statistically insignificant.

Here is another extreme case: macroscopical differences without any differentiation in important microscopical characters. Obviously *Ph. torulosus* is a younger species derived from a very old species *Ph. senex* by allopatric speciation. The Iranian Plateau and the Hindukush-Himalayan mountain ranges constitute a barrier between the two closely related species. The intermediate specimens found in the contact zone of these species are really intermediate and their species identity may be determined only arbitrarily. Presumably similar difficulties in species identification may occur in other groups of the Hymenochaetaceae as well, and no kind of methods will give us any help.

5.3 *Phellinus pini* (Brot.: Fr.) Ames and *Ph. chrysoloma* (Fr.) Donk

These two taxa have been already distinguished in Europe by several mycologists and forest pathologists as varieties, by others as subspecies and in the last few decades usually as species. There are noticeable differences in the basidiome habitus, spore size, cultural characters (including physiological differences), pathology, etc. In Europe *Ph. pini* grows mainly on *Pinus* and *Ph. chrysoloma* on *Picea*.

In India both the species have been treated as a single species *Phellinus (Fomes, Trametes) pini*. A number of valuable studies have been published on it in India (Bakshi *et al* 1970; Suri 1926; Bagchee and Bakshi 1950; Bakshi 1955; Thind *et al* 1970; Bakshi 1971). According to the descriptions published and the numerous herbarium specimens I have personally seen in several Indian herbaria, *Ph. pini sensu* of the Indian authors is actually *Ph. chrysoloma*. But there also exist some untypical herbarium specimens similar to those which I have collected in the Alatau Mountains in the Kazakh SSR (USSR). For practical questions of forest pathology it is desirable to study the taxonomy of these species in India using biometrical techniques, culture studies, etc.

In East Asia and North America there are no less than three taxa more of this cluster of microspecies conventionally named "*Phellinus pini* group".

5.4 *Phylloporia ribis* (Schum.: Fr.) Ryv. and *Ph. ephedrae* (Voronich.) Parm.

The spores of *Ph. ribis* are variable in different specimens and possibly there is a cluster of microspecies combined under the name of one species. Parmasto and Parmasto (1983) compared the specimens growing on the Rosaceae in Europe, in the Caucasus in Central Asia with the specimens growing in Central Asia, in the Caucasus and in South Europe on *Ephedra* and *Jasminum fruticans*. There are significant differences in the mean spore size: 3·35–3·9 × 2·65–3 μm in specimens from *Ephedra* and *Jasminum*, 2·9–3·35 × 2·4–2·65 μm in the specimens from the Rosaceae. The upper part of the context of the basidiome has a different structure in these taxa. In accordance with this, the new combination *Phylloporia ephedrae* (Voronich.) Parm. is proposed (basionymum: *Fomes ephedrae* Voronich., Ann. Mycol. **22**: 300, 1925) for the species growing on *Ephedra* and *Jasminum*.

Most of the species of *Phellinus* are more or less strictly specialized on the genera of trees and shrubs. The substrata of *Ph. ephedrae* are quite different from each other: *Ephedra* is a genus of the division Gymnospermae, *Jasminum* belongs to the Angiospermae. In Central Asia these shrubs are seen in many places growing near each other, and *Ph. ephedrae* once more demonstrates that speciation in the Hymenochaetaceae is mainly allopatric and not sympatric.

5.5 *Hymenochaete semistupposa* Petch and *H. yasudai* Imaz.

There are only two species with nodulose or submoniliform hyphidia in the hymenium of the genus *Hymenochaete*. *H. semistupposa* is found in Shri Lanka (type) and in the Uttar Pradesh State in India (Thind and Adalkha 1956); the data on its occurrence in Himachal Pradesh (Rattan 1977) are doubtful. It has also been found in South Africa (Natal), Kenya, Australia and New Zealand. *H. yasudai* has been found in Japan, China, in the southern part of the Primorskij Terr, and on Sachalin Island (USSR) on dead branches of coniferous trees.

The colour of the basidiomes of *H. yasudai* is variable, depending on the state of development of the hymenial layer. This is a common thing in the *Hymenochaete* and has led to the description of some species several times over as "new" species. The main difference between the two vicarious species lies in the better development of hyphidia (which look like a special kind of cystidia) and in larger spores (6·5–8·5 × 2–3 μm) in *H. yasudai* compared with *H. semistupposa* characterized by slightly and partly submonilioid hyphidia and smaller spores (3·5–4·2 × 1–2–(2·5) μm). The setae of the type of *H. semistupposa* are covered with a hyphal sheet, but this character has not been mentioned by other mycologists who have described this species (Cunningham, Talbot). The data on the spores of *H. semistupposa* are scarce and doubtful; the type has only a few old and damaged spores.

5.6 *Hymenochaete rheicolor* (Mont.) Lév. and *H. attenuata* (Lév.) Lév.

H. rheicolor (syn.: *H. tenuissima* (Berk.) Berk., *H. sallei* Berk. et Curt., *H. elegantissima* (Speg.) Sacc.) is a species widely distributed in the tropics and the subtropics (but not found in Africa and Australia). In India it has been mentioned by Léveille, Berkeley

and Curtis, Cooke and Lloyd, but afterwards found only in Darjeeling (Imazeki *et al* 1966). The types of *H. tenuissima* and *H. sallei* studied by us are sterile. This is a species worthy of a special study using new good herbarium material (spore prints are urgently needed!) and biometrical techniques: is there really only one species from Cuba to India and the Philippines?

H. attenuata is said to occur in India (Sikkim; see Imazeki *et al* 1966), in Malaya, Thailand, Indonesia, Australia, New Zealand and Japan. The descriptions published differ among themselves, one of the main characters (basidiomes with an attenuated base) is of little taxonomic value for the genus *Hymenochaete*. The only thing that indicates its possible independence from *H. rheicolor* is the area of the geographical distribution, which seems to be different.

There are a number of species, which were described in the previous century and which are similar (according to their poor descriptions) to the species mentioned above. The re-description of their types may help in solving nomenclatural problems, but it will be useless until a preliminary survey of the *Hymenochaete* species will be compiled based on numerous good herbarium (and living) specimens.

5.7 *Hymenochaete mougeotii* (Fr.) Cke. and *H. murashkinskyi* Pil.

"Typical" *H. mougeotii* is widespread on the bark of *Abies* in Europe, Siberia, Soviet Far East, China and Japan; in Europe it has rarely been found also on *Picea abies*. The basidiomes of this species are always resupinate.

H. murashkinskyi has been found on *Rhododendron* in Eastern Siberia, Soviet Far East and China; the basidiomes are effused-reflexed, its hymenium is sometimes layered, and the colour of the hymenium is a little bit different. However, all the specimens studied by us are sterile and the difference in colour may be due to the state of hymenium development.

What is "*H. mougeotii*" found in India? The data published by Rattan (1977) give little help. It has been collected on angiosperms (including *Rhododendron*), but the basidiomes are described as "resupinate to effused-reflexed". Australian and New Zealand *H. mougeotii* was described by Cunningham (1963) as closely adherent resupinate, the substrata indicated by him are all angiospermic. The spore size given by different authors varies widely, but due to the small spore size this may be (partly) due to errors.

This is again a case when the available specimens are usually sterile and the descriptions have been compiled without any special attention to possible differences (in spore size, in the structure of the hymenium, etc.) These data do not enable us to clear up the taxonomy of the species. Spore prints and biometrics may give some help in future.

6. Conclusions

For a taxonomical study of any fungal group a clear species concept and on its basis a practical standard of species are obligatory pre-requisites. This is of special importance when dealing with uniparental (homothallic) species. In this paper the author has tried to demonstrate the usefulness of the discrimination of sibling microspecies on the basis of the selection species concept.

Acknowledgements

The author expresses his gratitude to the USSR Academy of Sciences as well as to the UGC of India, which made it possible for him, by way of an exchange of scholars, to study the fungal flora of India in 1978/79. Thanks are also due to Prof. C V Subramanian, Prof K S Thind and Dr P S Rehill for the help given to acquaint himself with the fungal flora of India; the Directors of the herbaria of the Royal Botanic Gardens (Kew) and the Tokyo Science Museum to get fungal type specimens on loan; Miss R Aguraiuja for kind permission to use her unpublished data on the results of the incompatibility tests in *Phellinus*.

References

Bagchee K and Bakshi B K 1950 Some fungi as wound parasites of Indian trees; *Indian For.* **76** 244–253

Bakshi B K 1955 Diseases and decays of conifers in the Himalayas; *Indian For.* **81** 779–797

Bakshi B K 1971 *Indian Polyporaceae (On trees and timber)* (New Delhi: Indian Council of Agricultural Research)

Bakshi B K, Manjusri Sen and Balwant Singh 1970 Cultural diagnosis of Indian Polyporaceae. 2. Genera *Fomes* and *Trametes*; *Indian For. Rec.* N S **2** 245–276

Bas C 1977 Species-concept in *Amanita* sect. *Vaginatae*; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 79–103

Beaudry J R 1960 The species concept: its evolution and present status; *Rev. Can. Biol.* **19** 219–240

Bigelow H E 1977 Differentiation of species in *Clitocybe*; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 105–121

Bochanov V P and Lipshic C Ju 1955 On the problem of the size of species in higher plants; *Bot. Zh. (Moscow and Leningrad)* **40** 542–547 (Russ.)

Boidin J 1958 Essai biotaxonomique sur les Hydnés résupinés et les Corticiés; *Rev. Mycol. (Paris) Mém. hors-série* **6** 1–388

Boidin J 1971 Nuclear behavior in the mycelium and the evolution of the Basidiomycetes; in *Evolution in the higher basidiomycetes*, (ed.) R H Petersen (Knoxville: Univ. Tennessee Press) 129–148

Boidin J 1977 Interet des cultures dans la delimitation des especes chez les Aphyllophorales et les Auriculariales; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 277–239

Boidin J and Lanquetin P 1984 Répertoire des données utiles pour effectuer les tests d'intercompatibilité chez les Basidiomycetes. I. Introduction; *Cryptog., Mycol.* **5** 33–45

Burnett J H 1983 Speciation in fungi; *Trans. Br. Mycol. Soc.* **81** 1–14

Camp W H and Gilly C L 1943 The structure and origin of species; *Brittonia* **4** 323–385

Clémençon H (ed.) 1977 *The species concept in Hymenomycetes* (Vaduz: J Cramer)

Cunningham G H 1963 The Thelephoraceae of Australia and New Zealand; *Bull. N. Z. Dept. Sci. Ind. Res.* **145** 1–359

DeBach P 1969 Uniparental, sibling and semi-species in relation to taxonomy and biological control; *Israel J. Entomol.* **4** 11–28

Doll R 1982 Grundriss der Evolution der Gattung *Taraxacum* Zinn.; *Feddes Rep.* **93** 481–624

DuRietz G E 1930 The fundamental units of biological taxonomy; *Sven. Bot. Tidskr.* **24** 333–428

Ehrlich P R and Raven P H 1969 Differentiation of populations; *Science* **165** 1228–1232

Grant V 1957 The plant species in theory and practice; in *The species problem* (ed.) E Mayr (Washington DC: American Association for Advanced Science) 39–80

Grant V 1971 *Plant speciation* (New York: Columbia Univ. Press)

Grant V 1981 *Plant speciation*, 2nd edn (New York: Columbia Univ. Press)

Heywood V H 1963 The 'species aggregate' in theory and practice; *Regnum Veg.* **27** 26–37

Huxley J 1942 *Evolution. The modern synthesis* (New York: Harper and Brothers)

Imazeki R, Kobayashi Y and Aoshima K 1966 Fungi; in *The flora of Eastern Himalayas* (ed.) H Hara (Tokyo: Univ. Tokyo) 611–626

Kemp R F O 1977 Oidial homing and the taxonomy and speciation of Basidiomycetes with special reference to the genus *Coprinus*; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 259–276

Löve A 1964 The biological species concept and its evolutionary structure; *Taxon* **13** 33–45
Massee G 1891 A monograph of the Thelephoraceae.—Part II; *J. Linn. Soc., Bot.* **27** 95–205
Mayr E 1942 *Systematics and the origin of species* (New York: Columbia Univ. Press)
Mayr E 1948 The bearing of the new systematics on genetical problems: the nature of species; *Adv. Genet.* **2** 205–237
Mayr E 1957 Species concepts and definitions; adapted version in *Evolution and the diversity of life* E Mayr 1976 (Cambridge, Mass and London: Belknap Press of Harvard Univ. Press) 492–506
Mayr E 1963 *Animal species and evolution* (Cambridge, Mass: Harvard Univ. Press)
Mayr E 1969 The biological meaning of species; *Biol. J. Linn. Soc.* **1** 311–320
Parmasto E 1978 The genus *Dictyonema* ('Thelephorolichenes'); *Nova Hedwigia* **29** 99–144
Parmasto E and Parmasto I 1979 The xanthochroic reaction in Aphyllophorales; *Mycotaxon* **8** 201–232
Parmasto E and Parmasto I 1985 Variation of basidiospores in Hymenomycetes and its significance to their taxonomy; (in press)
Parmasto I and Parmasto E 1983 *Phellinus ribis* and *Ph. ephedrae* (Hymenochaetaceae); in *Materialy VI Zakavkazskoj konferencii po sporovym rastenijam* (Tbilisi: Acad. Sci. Georgian SSR) 94 (Russ.)
Pegler D N 1964 A survey of the genus *Inonotus* (Polyporaceae); *Trans. Br. Mycol. Soc.* **47** 175–195
Petersen R H 1977 Species concept in higher Basidiomycetes: taxonomy, biology and nomenclature; in *The species concept in Hymenomycetes*, (ed.) H Clémençon (Vaduz: Cramer) 363–380
Rattan S S 1977 *The resupinate Aphyllophorales of the North Western Himalayas* (Vaduz: J Cramer)
Romagnesi H 1977 Incidence des characteres non morphologiques sur la notion d' espece et autres taxa chez les macromycetes; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 349–361
Ryvarden L 1982 The genus *Hydnochaete* (Hymenochaetaceae); *Mycotaxon* **15** 425–447
Ryvarden L and Johansen I 1980 *A preliminary polypore flora of East Africa* (Oslo: Fungiflora)
Simpson G G 1961 *Principles of animal taxonomy* (New York: Columbia Univ. Press)
Slobodchikoff C N 1976 Introduction; in *Concepts of species* (ed.) C N Slobodchikoff (Stroudsburgs, Pa: Academic Press) 1–5
Snyder W C and Hansen H N 1954 Variation and speciation in the genus *Fusarium*; *Ann. New York Acad. Sci.* **60** 16–23
Sokal R R and Crovello T J 1970 The biological species concept: a critical evaluation; *Am. Nat.* **104** 127–153
Stafleu F A 1969 A historical review of systematic biology; in *Systematic biology* (Washington DC: National Academy of Sciences) 16–44
Stanley S M 1979 *Macroevolution. Pattern and Process* (San Francisco: W H Freeman)
Suri P N 1926 *Trametes pini* on deodar in the Baspa Valley, Bushahr State, Punjab; *Indian For.* **52** 327–330
Thind K S and Adlakha K L 1956 Thelephoraceae of Mussoorie Hills-I; *Indian J. Mycol. Res.* **2** 57–64
Thind K S , Rattan S S and Dhanda R S 1970 The Polyporaceae of India-VI; *Res. Bull. Panjab Univ.* N S **21** 109–117
Timofeeff-Ressovsky N W, Vorontsov N N and Yablokov A V 1977 *Short essay on the theory of evolution*, 2nd edn (Moscow: Nauka) (Russ.)
Turrill W B 1939 On subspecies and varieties; *Proc. Linn. Soc. London* **151** 90–95
Walters S M 1972 Endemism in the genus *Alchemilla* in Europe; in *Taxonomy, phytogeography and evolution* (ed.) D H Valentine (London and New York: Academic Press) 301–305
Watling R 1977 An analysis of the taxonomic characters used in defining the species of the Bolbitiaceae; in *The species concept in Hymenomycetes* (ed.) H Clémençon (Vaduz: J Cramer) 11–53
White M J D 1978 *Modes of speciation* (San Francisco: W H Freeman and Co.)
Wiley E D 1981 *Phylogenetics. The theory and practice of phylogenetic systematics* (New York: John Wiley)
Zavadskij K M 1968 *Species and speciation* (Leningrad: Acad. Sci. USSR) (Russ.)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 381–386.
© Printed in India.

The relevance of chemical characters for the taxonomy of Agaricales

MEINHARD MOSER

Institut für Mikrobiologie, der Universität Innsbruck, A-6020 Innsbruck, Sternwartestrasse 15, Austria

Abstract. Chemical characters prove to be more and more useful for the taxonomy of Agaricales. Nucleic acids and proteins have only sporadically been studied in Agaricales. Secondary metabolites, particularly pigments, however have been used in many families. Several examples are discussed, particularely from *Cortinarius*. The possibility to derive new ideas from correlations between chemical, mycogeographic and ecological characters is discussed.

Keywords. Chemotaxonomy; *Agaricales; Cortinarius; Dermocybe;* anthraquinonic pigments; styryl-pyrones.

Chemical characters are used more and more in taxonomic work on fungi in general, but also find application in Agaricales. In this group only recently several approaches have been made and some have proved to be quite useful, provided the evaluation has been made with the necessary skill and carefulness.

The basic chemical compound indicating genetic differences are nucleic acids. Several methods for comparison might be used as sequence analysis of specific genes or comparison of RNA molecules as rRNA. No examples however are known so far from Agaricales. An other way would be the relatively rough method by studying the DNA homology of different taxa. In the field of Agaricales I know only of one promising attempt in *Stropharia* (Jäger, oral communication).

The primary products of translation are proteins. For taxonomic purposes two aspects can be of interest. Specific proteins or the general pattern of proteins may be compared. Again only few examples are known from *Agaricales.*

Guillaume and Lamoure (1985) in a study of the *Armillaria mellea* group could demonstrate the presence of a specific protein in *A. bulbosa* (Barla) Rom., by which this species can be easily distinguished from other taxa of this group.

Serological tests (usually an indirect comparison of proteins) have been used for the identification of phytopathogenic or mycorrhizal fungi. In Agarics again not much work has been done in this direction. But Lung *et al* (1980) have demonstrated by such methods the presence of a specific protein (T_1) in *Armillaria mellea*, lacking in all other Armillaria species. Small amounts of the oligopeptide α-amanitine, which elude other methods of detection in several fungi, could be demonstrated by serological tests.

In the field of *Agaricales* ss. lat. and *Boletales* much more has been done with pigments. Pigments are secondary metabolites and one may ask how far they can serve as reliable characters. The question seems important whether their formation is genetically fixed and enzymatically catalyzed or whether more or less accidental pure chemical reactions are involved. This may differ with the type of chemical compounds.

The evaluation therefore needs much precaution and experience. A comparison of chromatographic fingerprints without any knowledge of the chemical background is of very restricted value, but can help to draw the attention to possible differences, which need further investigation.

The more we know about the chemical nature of the pigments involved and their possible biosynthetic pathway, the more can the taxonomist gain from such information. Only few examples can be cited here.

Boletales have been intensively studied by several groups of chemists and mycologists during the last two decades (numerous publications of Steglich *et al*, Bresinsky *et al*, Edwards *et al*).

The results have been very interesting and useful for the taxonomy of this group. (Bresinsky 1974; Besl *et al* 1975; Arpin and Kühner 1977). To a large extent they confirmed the existing taxonomic ideas and could give them more weight, but we owe also several new concepts to these researches, (*e.g.* the relationship of *Hygrophoropsis* and *Omphalotus*, (Bresinsky 1979).

Betalain pigments (derived from L-dopa) are another interesting example, existing in two quite unrelated groups, several *Amanitas* and in the bright coloured species of *Hygrocybe*. (Döpp and Musso 1973, 1974; Ardenne *et al* 1974).

Shortly mentioned may be also the pteridine pigments of *Russula*, connected with the riboflavin-metabolism. (Eugster 1973, 1977; Eugster *et al* 1970; Iten *et al* 1973).

The yellow styrylpyrones (*e.g.* hispidin, bisnoryangonin, hypholomines A and B, fasciculines A and B) (Gluchoff-Fiasson and Kühner 1977; Fiasson *et al* 1977) are characteristic of the genera *Pholiota, Hypholoma* and *Gymnopilus*. The first two belong, as accepted currently to the *Strophariaceae*, the latter to the *Cortinariaceae* having microscopic characters of the respective families. Is the chemical similarity sufficient to put them in the same family (*Strophariaceae*) as Kühner (1980) recently proposed? At a first glance this looks very plausible and a convergence in microscopic characters (spore morphology) cannot be excluded.

The existence of a South American *Cortinarius* species (*C. flammuloides* Mos. et. Hk.) containing hispidin however makes reconsideration necessary. If this remains the only *Cortinarius* with styrylpyrones the species can perhaps be transferred to *Gymnopilus*, although all its other characters point to *Cortinarius*. But at present the pigments of only a relatively small proportion of Cortinarii have been examined, particularly in the Southern hemisphere. But there is a further interesting case. In *Cortinarius renidens* Fr. occasionally pigments have been observed which eventually could be styrylpyrones, however different from the known ones. On the other side this *Cortinarius* contains leprocybin. Moreover the matter becomes more complicated by the fact that A H Smith described a *Gymnopilus terricola* which seems hardly distinct from *C. renidens* with respect to his macroscopic and microscopic characters. Chemistry may be helpful to settle these problems in the next future (Keller-Dilitz 1984).

Anthraquinonic pigments are a comparatively well studied group of pigments. They occur particularly in Dermocybe and some groups of *Cortinarius* and are known from two genera of tricholomataceous fungi.

Intensive studies of Steglich *et al* (1969, 1972), Töpfer-Petersen (1973), Steglich and Oertel (in press), Hofbauer (1983), Gruber (1970, 1975), Keller (1979), Keller-Dilitz (1984) and Brandrud (1980) accumulated a lot of information and today we have even some ideas of the biosynthetic pathway of different derivatives.

In these fungi neutral anthraquinones, anthraquinone carbonic acids and dimeric anthraquinoids can occur. If we consider the groups of *Cortinarius* containing such pigments, we find them in *Dermocybe*, in several sections of *Leprocybe*, in the sections Scauri and Fulvi of *Phlegmacium* and in few *Telamonias* (sect. *Armillati*). Many other groups of Cortinarii (*Myxacium, Sericeocybe, Paramyxacium*, most *Telamonia*, many sections of *Phlegmacium*) do not contain any pigments of this type.

Are the groups containing anthraquinonic pigments more closely related? Kühner (1980) proposed to unite *Dermocybe, Leprocybe* and the subgen. *Cortinarius* (*i.e.* the group around *C. violaceus*) in one subgenus *Cortinarius*. We have to object to this, as the type species of *Cortinarius, C. violaceus* (L.) Fr. is so different from the other groups both chemically and in microscopic features, that it seems impossible to unite it with *Dermocybe* and *Leprocybe* in one group. A close relationship of *Dermocybe* and part of *Leprocybe* (Hoiland 1983) seems more convincing at a first glance. But the data available now (Steglich (oral comm).; Keller-Dilitz 1984) seem to indicate a different biosynthetic pathway for the anthraquinones of Leprocybes *via* nonaketides (*via* oktaketides in *Dermocybe*). None of the pigments of Leprocybes are known from Dermocybes, moreover they contain xanthones in addition. This, together with other characters, might justify to keep *Leprocybe* separate as a subgenus, however not necessarily in the present circumscription (Keller-Dilitz 1984).

The position of the red-veiled Telamonias too needs a reconsideration. The pigments are identical or closely related with those of some groups of *Dermocybe* (Reininger *et al* 1972; Besl *et al* 1978). They may finally find their place in *Dermocybe* particularly when some south-hemispheric species are taken into account.

The situation is somewhat different when we compare *Dermocybe* and the bright coloured species of *Phlegmacium* sect. Scauri p.p. and sect. Fulvi, and may be, some other smaller groups. There we have several identical pigments and the same biosynthetic pathway. Of course, there are special developments and particularly interesting is a strong tendency to form dimeric anthraquinoids.

In *Dermocybe* we know only a few dimers, flavomannin-6,6′-dimethylether (known also in *Tricholoma* but with different gyration) in subgen. or sect. Dermocybe respectively, and skyrin an hypericin in subg. (or sect.) *Icterinula*. Skyrin is a relatively widely distributed pigment in fungi, hypericin has recently been found in the tricholomataceous genus *Callistosporium* (Moser, in press.) In *Phlegmacium* we know as yet one group (around *C. atrovirens*) containing skyrin, but many other dimeric anthraquinoids are known. These data invite to a taxonomic and systematic re-evaluation of several of the established infrageneric units in *Cortinarius*. It is certainly true that *Dermocybe* can be hardly maintained as a separate genus. But can we gain much by simply degrading it to a subgenus (Höiland 1983) or even a section of *Cortinarius* (Ammirati 1969)? I have more the impression that the data available now necessitate a redefinition, a new circumscription, eventually the creation of new infrageneric taxa. In the case of *Dermocybe* and *Phlegmacium* I see several possible solutions. In all cases one has to accept *Phlegmacium* as a heterogeneous subgenus, as the work of Hofbauer (1983) might suggest.

(i) To unite all anthraquinone containing species (except *Leprocybe*) in one subgenus *Dermocybe*.

(ii) To exclude *Icterinula* (or at least sect. Pauperae) from *Dermocybe* and unite it with the *Phlegmacia* of sect. Fulvi and Scauri (p.p.) in one subgen. *Icterinula* (if also the type

species of *Icterinula* can be transferred otherwise, we would need a new name).

(iii) To accept three subgenera, *Dermocybe* (under exclusion of *Icterinula* or part of it), *Icterinula* (ev. under inclusion of some species of *Phlegmacium*) and a third taxon (as yet no name would be available) comprising most of the Phlegmacia of sec. Fulvi and Scauri. The latter section would loose their name, as the group around *C. scaurus* would have to be excluded (Hofbauer 1983).

The present data are not sufficient to give more weight to any of these ideas, but the general development points to a solution in one of these directions. We have to reconsider other characters, whether there might be some correlation to chemical data, *e.g.* many species of Icterinula show a tendency of gelatinisation of the cap surface. And we need more information on the pigments in Phlegmacia of the southern hemisphere.

Also comparative studies on the geographical distribution of pigments and species and their mycorrhizal relations give interesting results (Moser 1972; Keller 1979). Until now such data are only available for *Dermocybe*. They show that we find the greatest number of species and pigments in the pacific area between Indonesia-Papua and New Zealand. In this area both subgenera (*Dermocybe* and *Icterinula*) overlap. Subgen. *Dermocybe* extends only down to New Zealand, Icterinula can be found also in the South American *Nothofagus* area (Patagonia, South Chile). If one accepts the hypothesis of van Steenis (1971), who proposes an origin of the *Fagales* in the area between Indonesia and Australia, we can accept a parallel evolution of *Dermocybe* with their host plants, where sg. *Icterinula* has migrated particularly with *Nothofagus*, sg. *Dermocybe* both with *Nothofagus* southward and with other *Fagales* to the northern hemisphere where it has acquired several new host genera including conifers. *Icterinula* must have reached South America during a period when a land connection existed *via* the antarctic area sg. *Dermocybe* has only reached New Zealand. This could mean, that sg. *Icterinula* is the older one. From the Northern hemisphere we know only one species, *Cort. olivaceofuscus* Kühn., which eventually can belong to *Icterinula*. It is associated with *Fagales* only. But here we cannot exclude as yet an interpretation as degradative form of the sg. *Dermocybe*, with the loss of several pigments.

Schuster (1976) proposed an origin of the *Fagaceae* in Laurasia during the middle cretaceous period. He accepted two lines of evolution, the one leading to North America, Europe and Siberia the other to South America and *via* the antarctic area to the Australian-Indonesian area, where both lines have met during the tertiary. If we consider the data available for *Dermocybe* they are hardly compatible with this hypothesis, but could be well explained with the hypothesis of van Steenis.

Are Icterinula-like species ancestors of both Phlegmacia and Dermocybes (sg. Dermocybe)? The tendency to gelatinise the cap surface and the occurrence of skyrin could point to a relationship between *Icterinula* and *Phlegmacium* (e.g. *atrovirens*-group), the occurrence of primitive anthraquinones is in accordance with *Dermocybe* sg. *Dermocybe* too.

Unfortunately we have no adequate data on Phlegmacia, all our information being restricted to European taxa. Many pigments in Phlegmacia are less stable and liable to oxidation. Thus dried material is not suitable. This made it impossible hitherto to extend the pigment analyses in Phlegmacia to the Southern hemisphere.

The proposed biochemical pathways of the pigments correlate fairly well with the frequency of pigments, the more primitive pigments as endocrocine, dermolutein etc.

being ubiquitous and present in most species, the substituted and more oxygenized ones becoming gradually rarer parallel with the degree of substitution, the most derived ones occurring only in few species, often in restricted areas.

Only some selected examples could be quoted here, but I think they obviously demonstrate the usefulness of chemical studies of fungi for taxonomic purposes. This can be for identification. Just in *Dermocybe* some taxa can hardly be determined from herbarium specimens only with the help of microscopic characters, but can easily be separated with the help of their pigment spectrum. Or in an advanced state having knowledge of the biosynthetic pathway correlation with plant geographical and ecological data may provide means to gain new ideas on evolutionary lines and this may be helpful for the definition of infrageneric units.

References

Ammirati J F 1969 Studies in the genus *Cortinarius* I. Sect. Dermocybe, *Cortinarius aureifolius* complex; *The Mich. Bot.* **8** 175–180

Ardenne R, Dopp H, Musso H and Steglich W 1974 Über das Vorkommen von Muscoflavin bei Hygrocyben (Agaricales) und seine Dihydroazapin Struktur; *Z. Naturforsch.* **C29** 637–639

Arpin N and Kühner R 1977 Les grandes lignes de la classification des *Boletales; Bull. Soc. Linn. Lyon* **46** 83–105, 181–208

Besl H, Bresinsky A and Kronawitter I 1975 Notizen über Vorkommen und systematische Bewertung von Pigmenten in Höheren Pilzen I; *Z. Pilzkd.* **41** 81–98

Besl H, Halbauer R and Steglich W 1978 Neue Anthrachinonfarbstoffe aus *Cortinarius armillatus* und *C. miniatopus* (Agaricales); *Z. Naturforsch.* **C33** 294–295

Brandrud T E 1980 En undersökelese av pigmentene i *Cortinarius*, underslekt *Phlegmacium* i: *Norden* 1–68, Diss. Oslo.

Bresinsky A 1974 Zur Frage der taxonomischen Relevanz chemischer Merkmale bei Höheren Pilzen; *Bull. Soc. Linn. Lyon.* No. Special p. 61–84

Bresinsky A 1979 Zum verwandtschaftlichen Anschluss von Omphalotus; *Sydowia, Beih.* **VIII** 98–109

Bresinsky A, Hilber O and Molitoris H P 1976 The genus *Pleurotus* as an aid for understanding the concept of species in Basidiomycetes in *The species concept in Basidiomycetes* (ed.) H Clemencon 229–258 (*Biblioth. Mycol.* 61)

Döpp H and Musso H 1973 Fliegenpilzfarbstoffe II. Isolierung der Farbstoffe aus *Amanita muscaria; Chem. Ber.* **106** 3473–3482

Döpp H and Musso H 1974 Eine chromatographische Analysen-methode für Betalainfarbstoffe aus Pilzen und Höheren Pflansen; *Z. Naturforsch.* **C29** 640–642

Eugster C H 1973 Pilzfarbstoffe, ein Überblick aus chemischer Sicht mit besonderer Berücksichtigung der Russulae; *Z. Pilzkd.* **30** 45–96

Eugster C H 1977 Pigments from *Russula* species: Abstr. 2nd Int. Myc. Congr. Tampa 178

Eugster C H, Frauenfelder E F and Koch H 1970 *Russula*-Farbstoffe; *Helv. Chim. Acta* **53** 131–138

Fiasson J L, Gluchoff-Fiasson K and Steglich W 1977 Über die Farb-und Fluoreszenzstoffe des Grünblättrigen Schwefelkvpfes (*Hypholoma fasciculare*, Agaricales); *Chem. Ber.* **110** 1047–1057

Gluchoff-Fiasson K and Kühner R 1977 La délimitation et la classification des *Strophariceae* à la lumiére des nouvelles recherches sur la structure des pigments; *C. R. Acad. Sci. Paris* **D284** 16067–16072

Gruber I 1970 Anthrachinonfabstoffe in der Gattung *Dermocybe* und Versuch ihrer Auswertung für die Systematik; *Z. Pilzkd.* **36** 96–112

Gruber I 1975 Papierchromatographische Pigmentanalvse von südamerikanischen Dermocyben und Cortinarien. In *Cortinarius Fr. u. verwandte Gattungen in Südamerika*, (eds.) M Moser and E Horak (Vaduz: Cramer) 521–540

Guillaume J J and Lamoure D 1985 The European species of *Armillaria* group Mellea; *Atti. Symp. Int. Tricholomataceae* (Borgotaro) (in press)

Höiland K 1983 *Cortinarius* subgenus *Dermocybe; Opera Bot.* **71** 5–113

Hofbauer C 1983 Chemotaxonomische Untersuchungen in der Untergattung *Phlegmacium*; Diss. **Innsbruck.**

Iten P X, Arihara S and Eugster C H 1973; *Helv. Chim. Acta* **56** 302
Keller G 1979 Chemotaxonomische Pigmentuntersuchungen in der Gattung *Dermocybe* (Fr.) Wünsche; Diss. Innsbruck
Keller-Dilitz H 1984 Chemotaxonomische Pigmentuntersuchungen in der Gattung *Cortinarius* Fr. Untergattung *Leprocybe* Mos; Diss. Innsbruck
Kühner R 1980 Les Hyménomycétes agaricoides: No. spéciale; *Bull. Soc. Linn. Lyon* **49** 1–1025
Lung-Escarmant B and Dunez J 1980 Les propriétés immunologiques, un critère possible de classification de l'Armillaire et des C litocybes; *Ann. Phytopathol.* **12**(1) 57–70
Moser M 1972 Die Gattung *Dermocybe* (Fr.) Wünsche (Die Hautköpfe): Schweiz; *Z. Pilzkd.* **50** 153–167
Moser M 1985 Notes on the genus *Callistosporium. Atti Symp. Inst. Tricholomataceae.* Borgotaro (in press)
Reininger W, Steglich W and Moser M 1972 Velumpigmente einiger Cortinarien der Untergattung *Telamonia* (Agaricales); *Z. Naturforsch.* **B27** 1009
Steglich W 1980 Pigments of higher fungi. (Macromycetes) In *Pigments in plants* (ed.) F C Czygan (Stuttgart: Fischer) 393–412
Schuster R M 1976 Plate tectonics and its bearing on the geographical origin and dispersal of Angiosperms In *Origin and early evolution of Angiosperms* (ed.) C B Beck (New York: Columbia Univ. Press)
Steenis C G G J van 1971 *Nothofagus*, key genus of plant geography, in time and space, living and fossil, ecology and phylogeny; *Blumea* **19** 65–98
Steglich W, Arnold R, Lösel W and Reininger W 1972 Biosynthesis of anthraquinone pigments in *Dermocybe; Chem. Commun.* **102**
Steglich W, Lösel W and Austel V 1969 Anthrachinonpiomente aus *Dermocybe sanguinea* (Wulf. ex. Fr.) Wünsche und *Dermocybe semisanguinea* Fr; *Chem. Ber.* **102** 4104–4118
Steglich W and Oetrel B Untersuchungen zur Konstitution und Verbreitung der Farbstoffe von *Cortinarius*, Untergattung *Phlegmacium* (Agaricales); *Sydowia* **37** (in press)
Töpfer-Petersen E 1973 Untersuchungen über die Farbstoffe von Phlegmacien; Diss. Berlin.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 387–406.
© Printed in India.

Monitoring of wheat rusts in the Indian sub-continent

L M JOSHI, K D SRIVASTAVA and D V SINGH

Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India

Abstract. Epidemiological studies of rusts of wheat were first taken up in India by Mehta who showed that due to intense summer heat the inoculum of rusts in any form is completely destroyed in the plains during the summer months. But the rust survives in the hills of North and South India. Recent work has identified different foci of infection and has shown that the primary source of stem rust lies mainly in the South Indian hills and that the North Indian hills contribute very little, if at all. Stripe rust, on the other hand, comes mainly from the northern hills while leaf rust is contributed both by southern and northern hills. This view is supported by detailed studies of temperature profile, incubation period, disease gradient etc. Moreover, it has been shown that the cyclones in the Bay of Bengal play a very vital role in dissemination of stem and leaf rusts from Nilgiri and Pulney hills. The ground survey data and information collected through rain sampler, satellite television cloud photography etc. are being utilized for developing bioclimatic models and linear prediction equations. For the disease management, erecting genetical barriers through gene-deployment has been suggested.

Keywords. Wheat rusts; epidemiology; directional movement; dissemination; predication; management.

1. Introduction

Wheat (*Triticum* spp.) constitutes a very important source of food to a vast population of many developing countries. In recent years some of these countries have registered a high growth rate (Rajaram *et al* 1984). In India no other major crop has achieved a growth rate in production comparable to wheat. The production of the country from nearly 7 million tonnes in 1948–49 has gone up to 45·5 million tonnes in 1983–84. Not only the production but the yield per unit hectare has also increased by almost three folds and today amongst wheat growing countries of the world India ranks 4th after USSR, USA and People Republic of China. However, there is still scope to enhance the productivity as vast areas of the country, suitable for wheat cultivation have not yet been fully exposed to modern wheat improvement technology. Pal (1978) has drawn attention to the fact that the production can be stabilized and increased to a great extent by mitigating the losses caused by diseases and pests. Swaminathan (1978) also felt that major problems faced by the scientists in stabilizing production of the dwarf varieties, is the reduction in genetic vulnerability to the major diseases of wheat in the sub-continent.

A major cause of instability of wheat production has been the rusts (stem rust = *Puccinia graminis* Pers. f. sp. *tritici* Erikss. & Henn. leaf rust = *P. recondita* Rob. ex. Desm. f. sp. *tritici* Erikss. & Henn. and stripe rust = *P. striiformis* West.). Wheat rusts have a long history of epidemics and have attracted the attention of plant scientists for a long time. A chronological account of the epidemics in India has been provided by Nagarajan and Joshi (1975) and Joshi *et al* (1980). Several workers have estimated the yield loss caused by rusts from time to time (Barclay 1890; Mehta 1940; Asthana 1948; Prasada 1960; Joshi *et al* 1975).

2. Early phase of rust research in India

The early research work of British Mycologists on rusts was restricted to taxonomical aspects and the role of alternate and collateral hosts in the annual recurrence of rusts. As early as in 1887 Barclay in India described Uredinales in the neighbourhood of Simla (western Himalayas) and recorded an aecidial stage on *Berberis aristata*, which he felt, could be of wheat rust. Barclay was not certain about the relationship of this aecial stage with wheat rust and therefore remarked, "this is identical with *P. graminis* as described by De Bary, though I have not confirmed its genetic relationship with *Puccinia* on cereals which occur very abundantly in all the fields around Simla. I am also not quite certain that all the three forms of *Berberis* which occur in Simla (*B. vulgaris, B. lycium* and *B. aristata*) bear the same species of aecidium. The subject is one which requires further investigations and I will, therefore, leave it at present an open question". Butler (1905) too had some doubts on the role of *Berberis* in the perpetuation of stem rust under Indian conditions. Butler's investigations were followed by detailed and systemic investigations on cereal rusts by Mehta from 1922–1950. He conclusively proved that the aecial stages occurring on *Berberis* spp. in the hills, are of no consequence in the perpetuation of stem rust in India (Mehta 1929, 1940, 1952). His views were further collaborated by subsequent workers. Prasada (1947) connected an aecial stage occurring on *Berberis* spp. with the rust on *Agropyron semicostatum*. Joshi and Payak (1963) established that the aecial stage on *Berberis jaeschkenana* in Lahaul valley of western Himalayas is connected with *Poa nemoralis*. Payak (1965) recorded that rust of *Brachypodium* has its aecial stage on *Berberis*.

Similarly it has been proved that *Thalictrum* species occurring in hills are non-functional as far as perpetuation of leaf rust is concerned (Mehta 1940). The aecial stage occurring on *Thalictrum javanicum* in Simla hills has been connected with brown rust (*Puccinia persistens*) of *Agropyron semicostatum* (Prasada 1946).

Mehta (1940, 1952) has shown that due to the high temperature prevailing in the plains of India during summer months and the following rainy season, wheat rusts in general, cannot survive while they could over summer in cooler climate of the hills on self sown plants, ratoon tillers and also on the regular summer crop of Nilgiri and Pulney hills. His studies proved that the chief source of infection of stem and leaf rusts are overwintering/oversummering of uredospores in the sub-Himalayan ranges particularly in central Nepal in the North and Nilgiri and Pulney hills in the South. Mehta (1952) also visualised the Sivalik ranges, the Hindukush mountain and the North-western Frontier Province (now in Pakistan) to be active foci in spreading the uredospores of leaf rust to the Indo-Gangetic plains. He felt that central Nepal is an active centre for spread of stem rust.

Mehta (1940, 1952) also studied the possible role of grasses in the annual recurrence of rusts and recorded the occurrence of *Puccinia graminis tritici* on *Brachypodium sylvaticum, Bromus patulus* etc. in the hills but could not establish any definite relationship. Later Prasada (1951), Vasudeva *et al* (1953), Lele and Rao (1961), Joshi and Manchanda (1963), Bahadur *et al* (1973) and Pathak *et al* (1979) tested a number of grasses against wheat rusts under glasshouse conditions and found a number of them to be susceptible. Some grasses like *Bromus coloratus, B. carinatus, B. mollis, B. patulus, Hordeum distichum, H. murinum, H. stenostachys, Lolium perenne, Brachypodium sylvaticum, Hilaria jamesii, Aegilops squarrosa, A. ventricosa, A. trinecilis* and *Bromus japonicus* were recorded to be susceptible to wheat rusts under natural conditions in the

plains and a few of them even in hills. However, the exact role of grasses in the perpetuation of wheat rust in the sub-continent needs more detailed investigations. It is worthwhile to mention here that in the Nilgiri and Pulney hills *Vulpia myuros* and *Briza minor* were thought to be the host of stem rust pathogen. Later on Joshi and Lele (1964) proved by cross inoculation experiments and race identification that the rust occurring on these grasses is *P. graminis avenae* and not *P. graminis tritici.*

Hardly any work on wheat rust epidemiology appears to have been done after 1940. Epidemiological studies were taken up only after 1968 when India ushered in the era of dwarf wheats.

3. Epidemiological studies in dwarf wheat era

The high yielding dwarf varieties, mainly carrying Norin-10 gene, were first introduced from Mexico in 1965 and their impact in Indian agriculture was felt only after 1967. The introduced wheat varieties, Sonora 64 and Lerma Rojo, though better yielders, did not possess the required level of rust resistance under Indian conditions. It was, therefore, felt in some quarters, that the newly introduced varieties might be totally destroyed by rusts and hence there is a need of close vigilence on the possible rust epidemics. With this aim in view, a National Wheat Disease Survey and Surveillance Programme was initiated in 1967 (Joshi 1975). This disease monitoring system has not only provided some very useful information on the performance of newly evolved varieties but also rejuvinated the interest in the epidemiological studies in India. Some results of recent epidemiological findings are discussed in the article.

3.1 *Stem rust epidemiology*

Mehta (1929, 1940, 1952) showed that stem rust can oversummers in the uredial stage on wheat either on the cultivated crop in Nilgiri and Pulney hills or self sown (volunteer) plants in the sub-Himalayan ranges, mainly in central Nepal. He considered northern hills, particularly central Nepal as "the most dangerous foci of infection". Recent investigations, however, are at variance with Mehta's conclusion about stem rust. It is now established that in case of stem rust, "the South Indian hills are the chief foci of infection and that the hills in North India contribute little, if at all, to stem rust epidemic in the main wheat belt" (Joshi *et al* 1971, 1974).

It is worthwhile to record that mobile survey-teams, in the last 16 years, have never recorded the incidence of stem rust in the foot hills or plains of Punjab, Himachal Pradesh, Haryana and western Uttar Pradesh before the middle of March (Swaminathan *et al* 1969; Joshi and Gera 1971; Joshi *et al* 1984b) while in certain pockets in the foot hills of North India such as Rupar, Gurdaspur (Punjab), Dhaula Kuaon (Himachal Pradesh) and Pantnagar (Uttar Pradesh), leaf and stripe rusts appear between middle of December and early January. Even Butler (1918) recorded that stem rust in North India does not appear on wheat until late in season. According to him it is often not seen until March, a time when wheat is in ears. If Himalayas were the active foci of infection for stem rust it should have normally appeared at the foot hills in western India at the most a fortnight later than leaf and stripe rusts. On the other hand stem rust appears as early as December/January in many places in peninsular India. This view that the bulk of inoculum of stem rust is introduced from South India was further substantiated by quantification of inoculum in North and South Indian hills by

Nagarajan and Joshi (1977). Using glass-rod impaction technique Roelfs *et al* (1968), found that the cumulate values of stem rust uredospores over naturally infected fields in southern hills have 20 to 25 times more uredospores in the air than the artificially infected field of North in the month of November (table 1).

The movement of stem rust from South to North has been well documented by ground survey data. Gokhle and Patel (1952) also reported similar movement of stem rust in erstwhile Bombay State. The present findings of Joshi (1976, 1978, 1982) and Nagarajan and Singh (1976) lead to the same conclusion for the whole country.

3.2 *Leaf rust epidemiology*

Leaf rust is the most important rust, widely distributed and most frequently occurring disease in the Indian sub-continent (Joshi *et al* 1970, 1984a). Somehow its importance has been overlooked in the past not only in India but elsewhere also (Chester 1946).

Mehta (1940, 1952) has shown that leaf rust spreads both from South and North Indian hills. Wheat disease surveys conducted since 1967 have also demonstrated that the first build up of leaf rust like stem rust takes place in the plains of Karnataka in South India, generally in the last week of December. At the same time the infection is also established in the foot hills of Bihar and eastern part of Uttar Pradesh. The rust population from southern foci moves Northwards towards Maharashtra and Madhya Pradesh and another population moves from the northern foot hills towards the South and finally both the populations, moving in opposite directions merge into each other (Joshi *et al* 1974).

Analysis of data, on rust appearance for over a decade and half, reveals that a number of foci get established all along the Himalayan foot hills, Bihar, East and central Uttar Pradesh between Christmas and middle of January. Such foci have been found up to 270 km from the nearest foot hills into parts of North east India (Joshi *et al* 1977). In the eastern region by mid January these isolated foci of the leaf rust infection multiply many times more while the isolated pockets of infection along the foot hills of Jammu

Table 1. Total number of stem rust uredospores caught over wheat fields of North and South India during the first four weeks of November 1973.

Location	No. of uredospores
South India	
Semabahnur	21
Coonoor	119
Wellington	94
Nanjnad	194
North India	
Palampur*	2
Solan*	0
Bhowali	6
Almora	12

* No infection recorded on wheat.

and Kashmir, Punjab, Haryana, Himachal Pradesh and western Uttar Pradesh remain more or less localised possibly because of non-congenial climatic reasons. In early February leaf rust is well established in the eastern regions of Indo-Gangetic plains and then the inoculum moves from central and eastern Uttar Pradesh towards North-west direction and rust appears uniformly allthrough western Uttar Pradesh and parts of Haryana. By the end of February with rise in average temperature, the less active infection centres along the foot hills of North-western India also become active and spread slowly and finally get mixed with the actively spreading population of uredospores from the East (figure 1). Thereafter, the two migrating populations are undistinguishable (Joshi *et al* 1977). Like stem rust the long distance dissemination of leaf rust from the southern foci to central India is reported to be associated with cyclonic rains (Nagarajan and Singh 1973, 1974).

3.3 *Stripe rust epidemiology*

Stripe rust has been found to survive during summer at several locations in the Himalayan ranges in the absence of alternate host (Mehta 1940). He reported that stripe rust could not be found throughout the year at higher altitudes like Narkanda in Simla hills (2850 m a.s.l.) due to very low temperatures. Joshi *et al* (1976a) have shown that there are probably very few foci which are capable of harbouring stripe rust all the year round but in general it survives within a range of 2200–2500 m a.s.l. or above, shifting up and down from one place to another as the environment changes. Joshi *et al* (1977) have further shown that normally this rust appears by the end of December and early January along the foot hills of Punjab, Haryana and western Uttar Pradesh and establishes primary foci of infection in this region as it can withstand cooler temperate conditions much better than other rusts. From this region, the stripe rust spreads

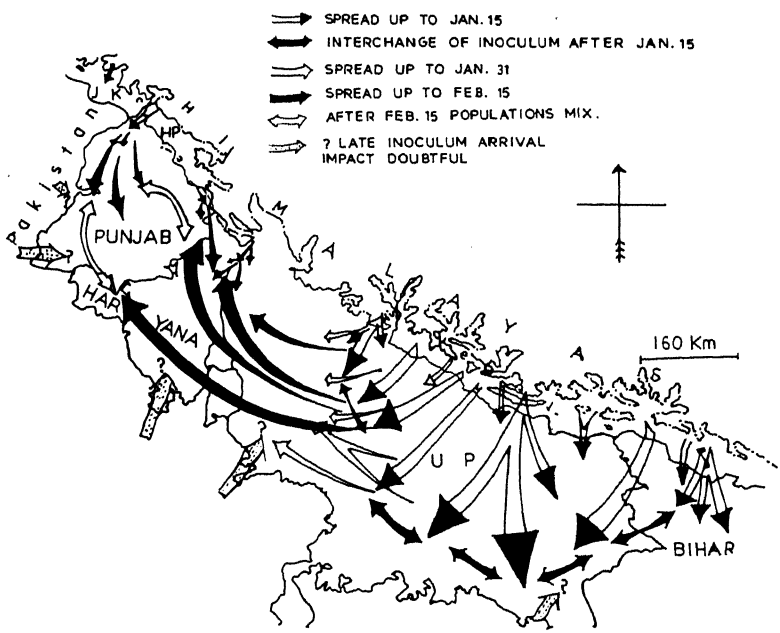


Figure 1. Spread of leaf rust in North-western India.

outhward and becomes well established in the northern parts of the country by the end of February.

During January and February the spread of stripe rust is much faster than leaf rust. However, by the end of February or beginning of March the temperature starts rising and further spread of rust stops and it develops into a telial stage. Therefore, the stripe rust remains essentially a major disease problem of North and North-western region (figure 1). Sometimes it also appears in Bihar, eastern Uttar Pradesh and even in central India (Joshi 1978). Very often these infections remain isolated and seldom become serious threat to wheat. The limited spread in South-central and eastern parts of the country is due to the prevalence of high temperature quite uncongenial for stripe rust development. The disease can survive in Nilgiri and Pulney hills but it cannot spread even to the foot hills of Nilgiris due to unfavourable weather. So far there is no evidence to suggest that stripe rust inoculum from Nilgiri hills is being introduced in the northern wheat belt (Joshi *et al* 1984a).

The present knowledge of stripe rust epidemiology is somewhat limited. Some question regarding the foci of infection need further elaboration. Is some inoculum in the North western Indian plains like Rajasthan (Joshi *et al* 1978) introduced from Pakistan also? What is the role of western mountains (Hindukush and Sulaiman ranges) in Pakistan itself? Yet another intriguing question, which remains unanswered is the appearance of a new virulent form of stripe rust in Nilgiri hills akin to Turkish virulence. Was it a case of 'introduction' or 'man guided evolution' as visualized by Zadoks (1959) and Johnson *et al* (1967) for similar cases elsewhere.

4. Climatic factors in relation to wheat rust epidemiology

4.1 *Temperature profile*

Temperature apparently is a major deciding factor in the development and spread of wheat rusts in the Indian sub-continent. Thorpe and Ogilvie (1961) also considered temperature as well as humidity to be the important climatic factors affecting the establishment and further growth of stem rust pathogen. In addition to this a free film of water on the leaf surface of the host influences the germination of uredospores and production of fungal hyphae (Rowell *et al* 1958; Sharp *et al* 1958). Temperature requirement for germination of spores of stem rust, appressorium formation and penetration are given in table 2.

Table 2. Temperature requirements for germination of stem, uredospores, appressorium formation, penetration and germ tube growth.

Conditions	Temperature (°C)		
	Minimum	Optimum	Maximum
Germination*	2	15–24	30
Penetration and sub-stomatal vesicle formation	15	29	35
Germ tube growth	—	20	—

* Free water necessary.

The temperature required for uredospore germination and penetration by germ tube leading to the formation of substomatal vesicle as indicated in table 2, are lacking in western Himalayas from November onwards. Joshi (1976) on the basis of 50 years meteorological data concluded that climatic conditions in the Himalayan region and even parts of North-western plains in winter months are not suitable for multiplication of stem rust inoculum. Therefore, he considered that stem rust inoculum surviving in Himalayas cannot be a source to Indo-Gangetic plains before middle of March and by then the crop is at an advanced stage.

Stem rust in northern hills probably remain dormant with almost no sporulation during winter months. This view gets support from an experiment conducted at Almora (5200 ft. a.s.l.) in 1972–73. Natural infection of stem rust, at Almora, was observed in October in a susceptible wheat cultivar. Immediately the same cultivar was sown surrounding it on all sides but the rust did not spread further in subsequent months and remained inert till late March. First pustule on the surrounding crop was seen on 26th March. This shows that the conditions for sporulation and subsequent spread of stem rust from the Himalayan region to the plain is not possible prior to March and then within 2–3 weeks the crop matures in the plains. Such late arrival of inoculum, if any, is hardly of any consequence. In all possibilities, the northern source of inoculum has very little role to play in the spread of rust to the plain to cause an epidemic.

As regards the effect of temperature on the spread of leaf rust, it has been noticed that North-eastern India is warmer in comparison to the North western parts in winter months (Nagarajan *et al* 1978b), and there is a gradual rise in temperature from North-east to North-west (table 3). Due to comparatively higher temperature and favourable climate in the eastern region in January and February there is a quick and fast multiplication of inoculum while the infection in the western region remains more or less inactive till middle of February on account of low temperatures. By the third or fourth week of February the foci established in the western region also become active mainly due to rising temperature, and rust begins to spread in the whole of the north-western plains.

Stripe rust can stand cooler temperatures much better than other rusts (its optimum being 9–11°C), therefore, it seems to form a closed circuit in North-west India. On the other hand, its absence practically from the entire South-central India except Nilgiri and Pulney hills, can be attributed to the prevalence of uniformly high temperatures

Table 3. Temperature profile in winter months in North-eastern and North-western region of India based on 30 years (1931–60).

	Average temperature (°C)					
	January		February		March	
Region	Min.	Max.	Min.	Max.	Min.	Max.
North-eastern zone (Bihar, east and central Uttar Pradesh)	9·8	23·5	11·9	26·5	16·9	32·79
Mid zone (Western Uttar Pradesh)	8·1	22·2	10·6	25·3	15·5	31·5
Western zone (Punjab and Haryana)	5·6	20·2	8·1	23·4	12·9	28·9

during crop season (table 4). The average minimum temperature in South India except hills seldom go below 15°C.

4.2 *Incubation period and rust development*

Incubation period is the time lapse between entry of the fungus into the host and the expression of first symptom. Low temperature greatly influences the establishment, development and further spread of the rusts. As reported by Stakman and Harrar (1957) the incubation period of stem rust can vary from 5 days to 3 months depending upon the prevailing temperature (table 5).

In India the incubation period in relation to rust development has been investigated by Mehta (1923), Sahni and Prasada (1963) and Pandey (1976). Sahni and Prasada (1963) reported the long interval up to 65 days between the establishment and first outbreak of stem rust infection and also reported that the incubation period of *P. graminis tritici* in the neighbourhood of Delhi was as long as 40 days. It is a bit puzzling (Johnson *et al* 1967). Pandey (1976) recorded that incubation period of stem rust varied from 22 days in January to 8–10 days during March at Delhi. Joshi *et al* (unpublished data) also reported that incubation period of stem rust in Delhi, in winter months was 15–18 days while in Simla (outer Himalayas) it ranged from 40–50 days, and this period was from 9–14 days in Indore in central India during crop season. Such a prolonged incubation period in the hills of North India, where low temperature persists from November to February, the multiplication rate of stem rust is either very low or almost nil. With the onset of congenial conditions in the northern hills after the

Table 4. Temperature range (in °C) for the hill stations of North and South India during winter months (Mean of 50 years).

	November		December		January		February		March	
Location	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
North India and Nepal										
Kathmandu	7·6	22·6	3·1	18·8	2·3	18·1	4·3	24·1	7·2	25·2
Mukteshwar	6·0	14·8	3·5	11·1	1·9	9·8	2·0	10·2	5·7	15·1
Nainital	5·3	15·6	2·2	11·0	1·4	9·6	3·4	13·3	9·0	18·0
Mussoorie	9·2	15·2	4·2	12·0	2·9	9·7	3·1	10·2	7·1	15·3
Simla	10·3	6·7	4·0	4·0	1·8	8·6	2·1	9·3	9·2	13.8
Dalhousie	7·7	17·1	3·7	12·0	1·8	8·7	4·7	13·6	9·2	17·2
Dharmsala	10·7	20·1	7·2	15·9	6·3	13·8	8·3	17·2	14·4	22·3
South India										
Kodaikanal	9·6	15·9	8·4	16·5	8·1	16·7	8·5	17·8	10·1	19·0
Ootacamund	8·9	17·6	8·8	19·7	6·1	18·8	6·6	19·7	8·8	21·1
Coonoor	11·8	19·4	10·7	20·5	8·4	19·0	9·5	21·0	11·2	22·7

Table 5. Incubation period for establishment and development of stem rust.

Temperature (°C)	0·5	5·5	10·5	12·2	19·1	21·1	23·8
Days	85	22	15	12	9	7	5+

middle of March some inoculum of stem rust, theoretically may be introduced from these hills but in North-western plains the remaining period is so short that rust cannot complete more than 1 or 2 uredial cycles till harvest time. In contrast to northern hills, the climatic conditions in Nilgiri hills in South are more uniform and the incubation period of stem rust is only 8–10 days in the cropping season. And many a times rust gets established in the foot hills and plains by middle of December. Moreover, climatic conditions in the entire South even in winter months are most congenial for stem rust development. Naturally huge amount of inoculum is present which actively multiplies from December to March in the whole of South while the northern hills are still under prolonged cold spells.

In case of leaf rust the incubation period varies from 10–25 days at Delhi in winter months (Sahni and Prasada 1968). Srivastava (1981) also observed that both at Delhi and Wellington (Nilgiris) weather conditions are congenial for rust development and therefore, the incubation period is more or less identical. The number of uredial generations calculated on the basis of incubation period revealed that it took 35–40 days for the completion of five uredial generations at Delhi and Wellington during crop season to build up leaf rust intensity to an epidemic level.

Disease gradient studies conducted at Karnal (altitude 2915 N and 74 D in North India) by Joshi and Palmer (1973) have shown that the weather in the month of January and February is generally too cool for stem rust development but the conditions, at that time, are favourable for leaf and stripe rusts development. It has been demonstrated that stripe and leaf rusts spread 100 and 92 m respectively from the source of inoculum in 55 and 75 days in 1970 and 1971 whereas stem rust spreads only 22 m in 1970 and 13 m in 1971 during the same period (figure 2). Joshi (1982), therefore, holds the view that even if stem rust is somehow established in northern parts in winter months it would either remain dormant or the rate of multiplication would be negligible till middle of March and by that time the crop is well in an advanced stage of growth.

4.3 *Role of cyclonic disturbances in the spread of rusts*

Sleeman (1839) was the first to correlate unusually wet weather due to heavy rains, with a severe epidemic of stem rust around Sagar in Madhya Pradesh during November, 1829. Moreland (1906) observed that the amount of rainfall in October is not a determining factor for rust development in Uttar Pradesh, but in Madhya Pradesh which is predominantly a stem rust sensitive area, the disease is influenced by rains. Mallik (1958) attempted to correlate stem rust epidemic with the unusual November rainfall in central India. It has now been proved that cyclonic disturbances in the Bay of Bengal have a direct bearing on the dissemination of uredospores of stem rust (and also leaf rust) from Nilgiri and Pulney hills to central India (Nagarajan 1973; Nagarajan *et al* 1975).

Nagarajan and Singh (1973, 1974) examined the rain samples collected in a rain sampler designed by Roelfs *et al* (1970) alongwith wind trajectories and satellite television cloud photography (STCP) and proved that uredospores of rusts from Nilgiris are lifted by conviction currents to 700 mb level and are transported to hundreds of kilometers towards North. In nature vertical mixing and small eddies occur which help to maintain the height of the spore cloud during transport (figure 3). Nagarajan and Singh (1975, 1976) suggested that the following synoptic weather conditions termed "Indian Stem Rust Rules" are associated with the transport of rust inoculum from Nilgiri hills to central India.

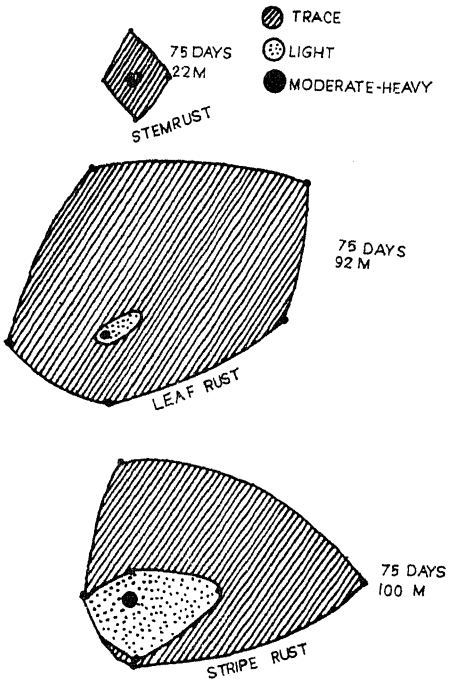


Figure 2. Wheat rust severity and distance from source 75 days after inoculation at Karnal, India, 1970

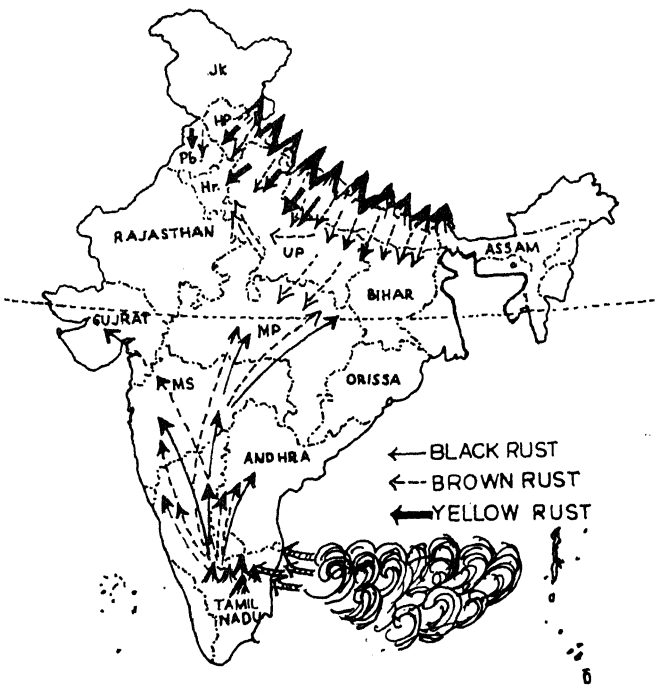


Figure 3. Dissemination of the three rusts from foci of infection.

(i) A storm of depression should be formed either in the Bay of Bengal or in the Arabian Sea between 75 and 85°E; 10–15°N and should end over central India.
(ii) A persistant high pressure cell over the southern part of central India (not far from the Nilgiris) must be present.
(iii) Appearance of a deep trough extending up to southern India and caused by onward movement of the easterly disturbance.

Many trajectories have been plotted and a number of cases of rust appearance have been investigated (Nagarajan *et al* 1976, 1977). These have conclusively shown that the cyclones were directly responsible for stem rust appearance in central-peninsular India. A typical case study of stem rust appearance in central and peninsular India during 1976 crop season is discussed here. It showed that synoptic weather conditions (*i.e.* the formation of a storm of depression in the Bay of Bengal, and the presence of high pressure cell in central India prevailed for more than 48 hr over central India (figure 4a). The backward trajectories drawn for Powerkheda, Indore and Malegaon (figure 4b) ended very close to the source area in the Nilgiri in about 56 hr. Resultantly the disease appeared in many parts of Madhya Pradesh and Maharashtra (figure 4c) as per expectation (Joshi *et al* 1976b; Nagarajan and Joshi 1980).

The correlation of the weather situation on the ground by visible and IR spectrum has shown that a more quantitative assessment of the weather is possible by IR images (Nagarajan *et al* 1982a, b). The IR images were superior to normal ones as they show more clearly the areas where the cyclone is dissipated. Results indicate that weather satellite scans in IR spectrum of light have some scope as a technique for predicting crop health.

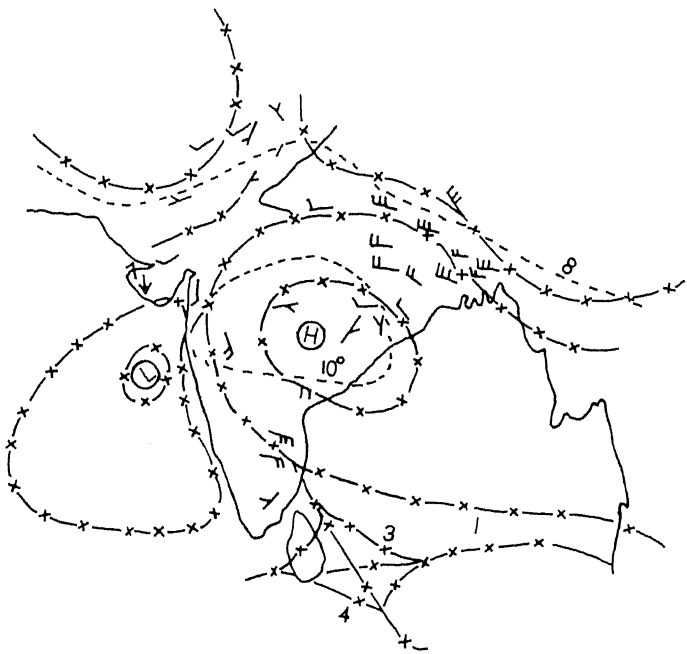


Figure 4a. Synoptic map for 700 mb level for one of the days (23rd 0000 GMT) when southerly winds prevailed due to tropical cyclone in November, 1976.

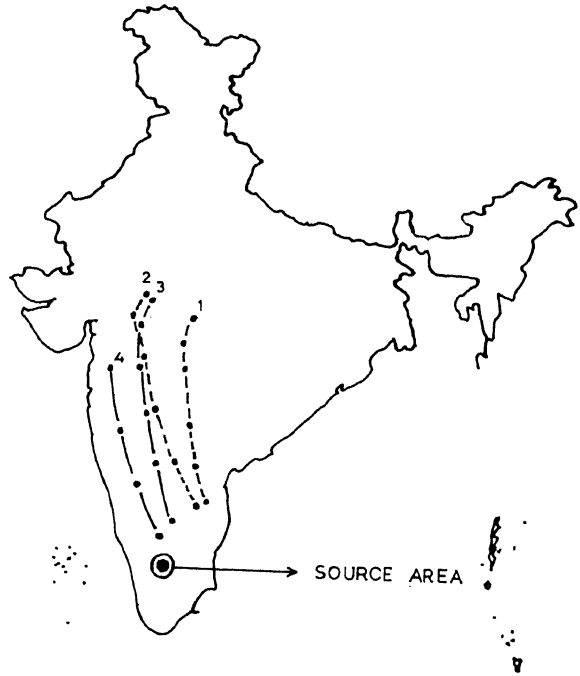


Figure 4b. Backward drawn 700 mb level trajectories for the rain deposited uredospores sampled at **1.** Powerkheda **2–3.** Indore and **4.** Malegaon for November 1976.

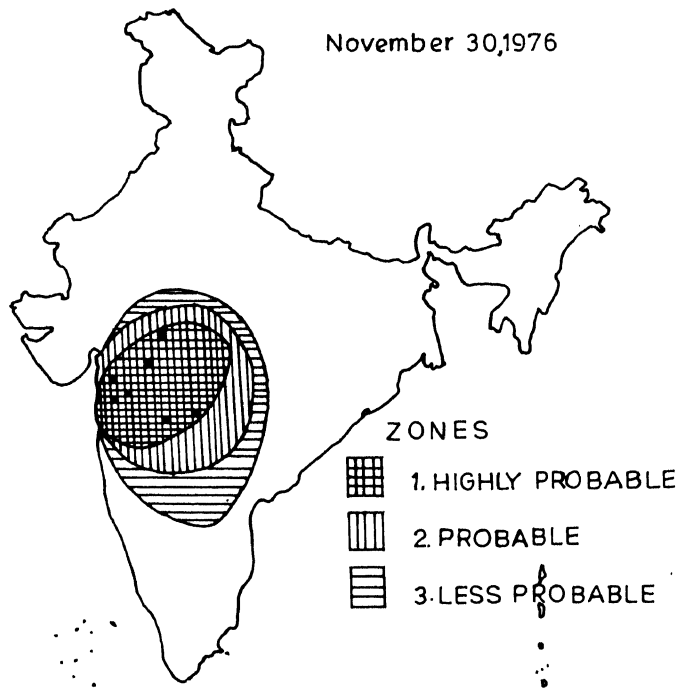


Figure 4c. Early November prediction of rust appearance. Dark dots—places where stem/leaf rusts appeared as predicted.

Like stem rust, the long distance dissemination of leaf rust from southern foci to central India is also associated with cyclonic rains (figure 3). Investigations have shown that a spread occurs to a distance of 600 km or more in central India from Nilgiri and Pulney hills without infecting the fields in between (Nagarajan and Singh 1973, 1974; Joshi 1976). Furthermore, it has been shown that the uredospores of leaf rust from North-eastern region are carried westwards by western disturbances. It has also been shown that the amount of precipitation and number of rainy days are more in epidemic than non-epidemic years (Nagarajan and Joshi 1978a). Keeping in view these conditions, they concluded that if more western disturbances accompanied by frequent rains occur in North India, there is apparently good chance for the spread and build up of leaf rust in North-west India to lead to an epidemic (Nagarajan *et al* 1978b).

A severe epidemic of stripe and leaf rusts occurred in some parts of Pakistan during 1978 (Hassan 1978; Kidwai 1979). In the absence of the daily weather report of Pakistan, Nagarajan *et al* (1980, 1982a) analysed weather data of Ganganagar, Ferozepur, Amritsar, Ludhiana, Jammu and Srinagar along the Indo-Pakistan border and found that crop season 1978 was very cool, wet and had an extended winter favourable for generation of more uredial cycles. Towards early April there was sudden rise in maximum temperature by 5–6°C. Such weather patterns were associated with western disturbances, which were congenial for rusts epidemic. It is presumed that similar climatic conditions prevailed in Pakistan region and due to the availability of susceptible cultivar (Maxipak, Chenab 70, Punjab 76 etc.), a severe epidemic occurred. On the other hand in the Indian side, the susceptible variety Kalyansona (a sister strain of Mexipak) had been largely replaced after 1973 by a number of varieties like Sonalika, WL 711, WG 357 etc. and this mosaic pattern of varieties averted the possible catastrophy.

5. Management strategies

5.1 *Rust prediction models*

Disease prediction is of fundamental importance for successful and efficient use of suitable chemicals for the management of rusts. The ground survey data and the information collected through rain samplers, STCPS etc. are being used as basis for developing bio-climatic models as well as linear prediction equations. Multiple regression analysis of various environmental factors has been used by several workers to work out mathematical equations for wheat rusts (Dirks and Romig 1970; Eversmeyer and Burleigh 1970; Eversmeyer *et al* 1973; Burleigh *et al* 1972; Kranz 1974).

As pointed out earlier, the cyclonic disturbances in the Bay of Bengal to a great extent are responsible in the dissemination of stem rust in India. A bio-climatic model has been developed to predict stem rust appearance in central India based on stem rust rules (Nagarajan and Singh 1976; Nagarajan 1977). On the basis of the cyclonic rains coupled with southerly winds during November, availability of susceptible cultivars and the detection of uredospores in rain samples some successful predictions were made on the probability of rust appearance during the years of cyclones. It was observed that between 1974–75 to 1983–84 seasons, cyclones over foci of infection occurred during 1976–77, 1977–78 and 1978–79 seasons. It is interesting to note that the disease appeared with wide prevalence during the years, of cyclonic disturbances, than in non-cyclonic years between 1980–81 to 1983–84 crop seasons which proved

the validity of prediction system based on rain analysis technique (Joshi *et al* 1984a).

Like stem rust some common feature on the development of leaf rust in North India, occurring every season have also provided the basis of bio-climatic prediction model in North-western India (Nagarajan *et al* 1979b). This model indicates that the following criteria must be satisfied if leaf rust is to occur:

(i) At least 5–6 infection sites should be observed at a maximum distance of 25 ± 5 km around 15–20 January in Uttar Pradesh and North Bihar.
(ii) The number of rainy days between January to middle of April over North-western India should be atleast twice the normal number.
(iii) The weekly mean maximum temperature during March to middle of April should be within 1°C of the normal temperature.

It has been assumed that if the susceptible cultivar in the entire Indo-Gangetic plain are cultivated (although in reality they are not) and 1st and 2nd criteria are satisfied then a severe epidemic will tend to occur. If they are partly satisfied, isolated outbreaks may probably occur. The testing of this model is under way.

The bio-climatic model can give an idea of probable dates of rust appearance but further development of the disease will depend on environmental factors prevailing after the establishment of infection. In this direction Nagarajan and Joshi (1978b) attempted to develop a mathematical model for a seven day forecast of stem rust severity based on environmental variables. The linear equation outlines as under:

$$\hat{y} = -29{\cdot}3733 + 1{\cdot}820\,x_1 + 1{\cdot}7735\,x_2 + 0{\cdot}2516\,x_3$$

where x_1, x_2 and x_3 were previous disease severity, minimum temperature and maximum relative humidity.

The above linear model has been tested and found valid for prediction at Delhi and Niphad under natural conditions of stem rust infection (Karki *et al* 1979).

A similar study was undertaken by Srivastava (1981) to evolve a functional equation for seven day prediction of leaf rust. The prediction equation being

$$\hat{y} = -83{\cdot}53084 + 1{\cdot}55172\,x_1 + 0{\cdot}54158\,x_2 + 1{\cdot}75326\,x_3$$

where x_1, x_2 and x_3 were mean of weekly maximum temperature, relative humidity and previous level of disease severity.

These mathematical models may be useful in estimating the severity of the disease a week before under local conditions, thereby giving enough time to decide, the economic use of chemicals for the disease management.

5.2 *Genetic barriers*

The epidemiology of rusts has an important bearing on their management in India. The efficiency of gene combination shows that the management of rusts may be possible by deploying the resistance genes (Nagarajan 1984). Gene deployment visualised a centrally planned, properly executed strategic use of vertical resistance genes over a large area to minimise the threat of rust epidemics or pendemics (Joshi and Nagarajan 1978; Nagarajan and Joshi 1984). This strategy can be quite useful in the case of pathogen where its epidemiology has been studied in depth and an organised machinery exists for seed distribution.

Nagarajan and Joshi (1980) considered the whole Indian sub-continent to be a single

epidemiological zone for stem rust which can be split into three sub-zones. They have also defined the "Puccinia Path" *i.e.* the usual dissemination pattern of the spread of inoculum from foci of infection (figure 5). According to them sub-zone 1, by virtue of its proximity to the source-area, gets inoculum by means other than rain such as katabatic wind current. This sub-zone, however, is not of much economic significance as the wheat acrage in this area is negligible. In sub-zone 2, the inoculum arrives either by wind current or with rain around November while in sub-zone 3 the inoculum is transported by cyclonic disturbances and deposited by rain during late October to November. This zone is epidemiologically important because the upper air transportation and rain deposition of uredospores can create an epidemic. This zone can also serve as the main secondary focus for zone 4. If there is quick and early built up of inoculum in this zone sometime in November or early December it can provide adequate inoculum to the eastern regions of sub-zone 4. Sub-zone 4 remains almost free from stem rust in the absence of an appropriate tropical cyclone.

This basic information on dissemination of rust can be utilized in erecting suitable genetic barriers in "Puccinia Path" by effective gene deployment to mitigate the rust menace. It should be possible, to delay the spread of the rust disease in the major wheat area by obstructing the "Puccinia Path" by growing genetically different resistant material in different zones. This sort of gene deployment could be practised at regional and national level (Swaminathan 1978). Similar recommendations have been proposed

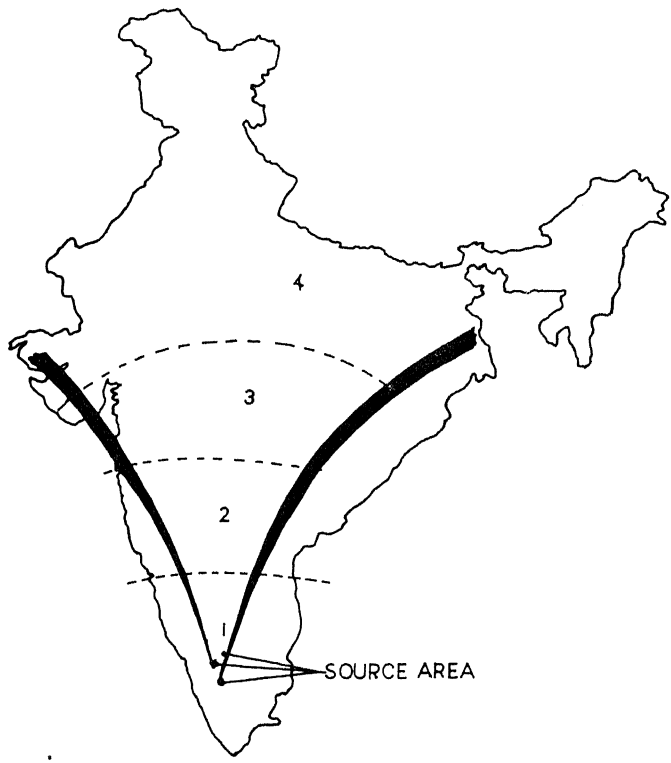


Figure 5. The 'Puccinia Path' as it occurs in India between the southern hills (darkly shaded) to central India and its sub-epidemiological zones 1–4.

for obstructing "Puccinia Path" of crown rust of oat in USA by deploying different vertical genes by Browning *et al* (1969).

The epidemiology of leaf rust in India reveal that the Indo-Gangetic plains and sub-Himalayan ranges act as a single epidemiological unit for this rust. This region can further be divided into four zones (figure 6) mentioned below (Swaminathan 1978; Nagarajan *et al* 1979).

Zone 1	– Eastern zone	– Bihar and eastern Uttar Pradesh
Zone 2	– Mid zone	– Western Uttar Pradesh
Zone 3	– Western zone	– Punjab, parts of Haryana and Himachal Pradesh.
Zone 4	– Himalayan zone	– Hilly region of Himalayas and mid-low altitudes of central Nepal.

In Zone 1, not only leaf rust gets established quite early but the multiplication rate of inoculum of the rust is also quicker and faster than the western zone with the result that by middle of February enough inoculum is build up in this zone, which is then transported to the western zone by easterly winds. It is, therefore, obvious that by checking this inoculum load coming from the eastern zone in February, the chances of epidemic can significantly be minimized. It can be achieved by growing highly resistant varieties with different genetic make up than those for the western zone. Such diversification of varieties will prevent introduction of matching genes from one zone to another. Survey and meteorological data between 1973–1978 have shown that by such varietal diversification in the Indo-Gangetic plains, a possible calamity of rust epidemic

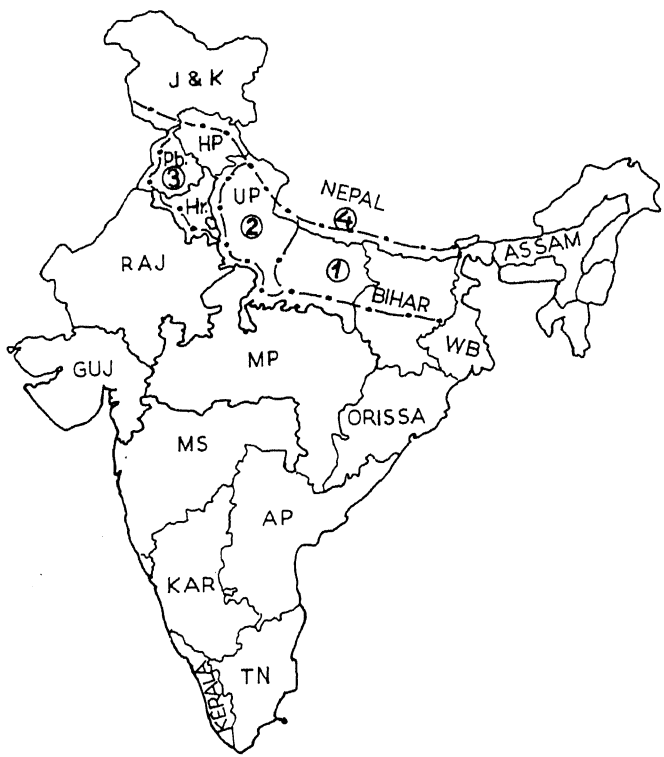


Figure 6. Sub-epidemiological zones of leaf rust in northern India.

in 1976–77 was prevented. It could be possible because the predominant cultivar Kalyansona possessing *Lr* 13, *Lr* 14b and *Lr* 18 genes in Indo-Gangetic plains had already been replaced by wheat cultivars such as Sonalika (*Lr* 2a, *Lr* 11, *Lr* 13), Arjun (*Lr* 10), WG 357, WG 377 and C 306 during this period (Nagarajan *et al* 1978a). A varietal change every 4–5 years in eastern and western zones with varieties possessing diverse gene for resistance has been recommended for leaf rust control in North India (Joshi and Nagarajan 1978; Nagarajan *et al* 1979). Nagarajan *et al* (1984) considered that there is scope for gene deployment on national level to check the spread of leaf rust. Srivastava *et al* (1984a,b) have explored the possibility of deployment of multilines in the eastern zone to mitigate the leaf rust epidemics.

From 1967 to 1975 the stripe rust remained very much restricted in the Indo-Gangetic plains due to extensive cultivation of Kalyansona, than a resistant cultivar (Joshi 1976). Kalyansona was withdrawn from cultivation in North-western India due to stripe and leaf rusts epidemic of 1971–72 and 1972–73 and was replaced by other resistant cultivars such as WL 711, Sonalika etc. Sharma *et al* (1973) has reported that Sonalika has now become susceptible to new stripe rust virulence (Race I) but for some unknown reasons the race has not spread much in the last decade. Anyway it poses a threat to Sonalika. Moreover, Sonalika is quite susceptible to a few races of leaf rust also. It is, therefore, essential to replace Sonalika by new genotypes in North-western region.

References

Asthana R P 1948 Wheat rusts and their control; *Mag. Agric. Coll. Nagpur* **22** 136–143

Bahadur P, Singh S, Goel L B, Sharma S K, Sinha V C, Ahmad R U and Singh B P 1973 Impact of grass introduction on cereal rusts in India; *Indian J. agric. Sci.* **43** 287–290

Barclay A 1887 A descriptive list of uredinales occurring in the neighbourhood of Simla (western Himalayas); *J. Asiat. Soc. Beng.* **56** (Part II) 350–375

Barclay A 1890 On some rusts and mildews in India; *J. Bot.* **20** 257–261.

Browning J A, Simons M D, Frey K J and Murphy H C 1969 Regional deployment for conservation of oat crown rust resistance. In: *Disease Consequences of Intensive and Extensive Culture of Field Crops*, Iowa agric. home Econ. exp. Sta. spec. Rep. **64** 49–56

Burleigh J R, Roelfs A P and Eversmeyer M G 1972 Estimating damage to wheat caused by *Puccinia recondita tritici; Phytopathology* **62** 944–946

Butler E J 1905 Some Indian forest fungi, *Indian Forester* **31** 670

Butler E J 1918 *Fungi and diseases in plants*; Thacker Spinck and Co., Calcutta pp 547

Chester K S 1946 *The cereal rusts, the nature and prevention of the cereal rusts as examplified in the leaf rust of wheat*; Chronica Botanica Co. Weltham, Mass. pp. 269

Dirks V A and Romig R W 1970 Linear model applied to variation in number of cereal rust urediospores; *Phytopathology* **60** 246–251

Eversmeyer M G and Burleigh J R 1970 A method for predicting epidemic development of wheat leaf rust; *Phytopathology* **60** 805–811

Eversmeyer M G, Burleigh J R and Roelfs A P 1973 Equation for predicting wheat stem rust development; *Phytopathology* **63** 348–351

Gokhale V P and Patel M K 1952 Suggestions for reducing epidemics of black stem rust of wheat in peninsular India; *Poona agric. Coll. Mag.* **42** 247–252

Hassan S F 1978 Rusts problem in Pakistan p 90–93, In: *Wheat research and production in Pakistan* Vol I (ed) M Tahir, PARC Islamabad, Pakistan

Johnson T, Green G J and Samborski 1967 The world situation of the cereal rust; *Ann. Rev. Phytopathol.* **5** 183–200

Joshi L M 1975 Surveys on wheat rusts in India: The rust situation since 1972; *Cereal Rusts Bull.* **3** 7–9

Joshi L M 1976 Recent contributions towards epidemiology of wheat rusts in India; *Indian Phytopath.* **29** 1–16

Joshi L M 1978 Dissemination of wheat rusts in the Indian sub-continent; *Botanical Progress* **1** 1–5

Joshi L M 1982 Wheat rust management—present knowledge and future prospects; *Kavaka* **10** 1–12

Joshi L M and Gera S D 1971 *Wheat Disease News Letter IARI New Delhi* **5** 1–8

Joshi L M and Lele V C 1964 Role of *Vulpia myuros* and *Briza minor* in the perpetuation of black rust of oats, in the Nilgiri Hills; *Indian Phytopath.* **17** 245–248

Joshi L M and Manchanda W C 1963 *Bromus japonicus* Thumb. susceptible to wheat rusts under natural conditions; *Indian Phytopath.* **16** 312–313

Joshi L M and Nagarajan S 1978 Regional deployment of *Lr* genes for brown rust management, In: *Genetics and Wheat Improvement* (ed) A K Gupta (Oxford & IBH Publishing Co: New Delhi) p 268

Joshi L M and Palmer L T 1973 Epidemiology of stem, leaf and stripe rusts of wheat in northern India; *Plant Dis. Reptr.* **57** 8–12

Joshi L M and Payak M M 1963 A *Berberis* aecidium in Lahaul valley Western Himalayas; *Mycologia* **55** 247–250

Joshi L M, Renfro B L, Saari E E, Wilcoxson R D and Raychaudhuri S P 1970 Rust and smut diseases of wheat in India; *Plant Dis. Reptr.* **54** 391–394

Joshi L M, Saari E E and Gera S D 1971 Epidemiological aspects of *Puccinia graminis* var. *tritici* in India. *Proc. Indian Nat. Sci. Acad.* **37B** 449–453

Joshi L M, Saari E E, Gera S D and Nagarajan S 1974 Survey and epidemiology of wheat rusts in India. In: *Current trends in plant pathology* (eds) S P Raychaudhuri and J P Verma pp 150–159

Joshi L M, Srivastava K D and Ramanujam K 1975 An analysis of brown rust epidemics of 1971–72 and 1972–73; *Indian Phytopath.* **28** 138

Joshi L M, Goel L B and Sinha V C 1976a Role of Himalayas in the annual recurrence of yellow rust in northern India; *Cereal Rust Bull.* **4** 27–30

Joshi L M, Srivastava K D, Nagarajan S and Singh D V 1976b *Wheat Disease News Letter* **9** 1–6

Joshi L M, Nagarajan S and Srivastava K D 1977 Epidemiology of brown and yellow rusts of wheat in North India I. Place and time of appearance and spread; *Phytopath. Z.* **90** 116–122

Joshi L M, Srivastava K D, Singh D V, Nagarajan S and Goel L B 1978 Wheat disease survey in India during 1975–76; *Annual Wheat News Letter, USA* **24** 60–61

Joshi L M, Srivastava K D, Singh D V and Ramanujam K 1980 Wheat rust epidemics in India since 1970; *Cereal Rust Bull.* **8** 17–21

Joshi L M, Singh D V and Srivastava K D 1984a Fluctuations in the incidence of rusts and other wheat diseases during past decade and strategies for their containment; *23rd All India Wheat Research Workers Workshop* (ICAR) held at Kanpur Aug. 1984

Joshi L M, Srivastava K D and Singh D V 1984b *Wheat Diseases News Letter* **17** 1–120

Karki C B, Pande S, Thombre S B, Joshi L M and Nagarajan S 1979 Evaluation of a linear model to predict stem rust severity; *Cereal Rusts Bull.* **7** 3–7

Kidwai A 1979 Pakistan reorganises agricultural research after harvest disaster; *Nature* **227** 169

Kranz J 1974 *Epidemics of plant disease—Mathematical analysis and modelling* (ed) Ecological studies 13, (New York: Springer Verlagh) pp 161

Lele V C and Rao M H 1961 Further studies on the susceptibility of some grasses to cereal rusts; *Indian Phytopath.* **14** 154–159

Mallik A K 1958 An examination of the crop yields at crop weather stations with special reference to rainfall; *Indian J Mat. Geophys.* **9** 1–8

Mehta K C 1923 Observations and experiments on cereal rusts in neighbourhood of Cambridge with special reference to their annual recurrence; *Trans. Brit. mycol. Soc.* **8** 124–176

Mehta K C 1929 Annual recurrence of rusts on wheat in India; *Proc. 16th Indian Sci. Congr.* pp 199–223

Mehta K C 1940 Further studies on cereal rusts in India. Part I. *Sci. Monogr. Imperial Coun. agric. Res. India* **14** pp 224

Mehta K C 1952 Further studies on cereal rusts in India. Part II. *Sci. Monogr. Indian Coun. agric. Res. India*, **18** pp 363

Moreland W H 1906 The relation of weather to rust on cereals; *Mem. Dep. agric. Indian Bot.* **1** 53–57

Nagarajan S 1977 Meteorology and forecasting of epidemics of diseases; In: *Symp. Basic Sciences and Agriculture*; Proc. INSA, New Delhi pp 136–143

Nagarajan S 1984 Disease problems and their management. In: *Global aspects of food production* (eds) M S Swaminathan and S K Sinha (New York: Academic Press)

Nagarajan S and Joshi L M 1975 A historical account of wheat rust epidemics in India and their significance; *Cereal Rusts Bull.* **3** 29–33

Nagarajan S and Joshi L M 1977 Sources of primary inoculum of wheat stem rust in India, *Plant Dis Reptr.* **61** 454–457

Nagarajan S and Joshi L M 1978a Epidemiology of brown and yellow rusts of wheat over North India. II. associated meteorological conditions; *Plant Dis. Reptr.* **62** 186–188

Nagarajan S and Joshi L M 1978b A linear model for seven day forecast of stem rust severity; *Indian Phytopath.* **31** 504–506

Nagarajan S and Joshi L M 1980 Further investigations on predicting wheat rusts appearance in central and peninsular India; *Phytopath. Z.* **98** 84–94

Nagarajan S and Joshi L M 1984 Epidemiology in the Indian sub-continent. In: *Cereal Rusts* Vol II (eds) A P Roelfs and Bushnell (New York: Academic Press)

Nagarajan S and Singh H 1973 Satellite television cloud photography as a possible tool to forecast plant disease spread; *Curr. Sci.* **42** 273–274

Nagarajan S and Singh H 1974 Satellite television cloud photography—a new method to study wheat rust dissemination; *Indian J. Genet.* **34A** 486–490

Nagarajan S and Singh H 1975 The Indian stem rust rules—an epidemiological concept on the spread of wheat stem rust; *Plant Dis. Reptr.* **59** 133–136

Nagarajan S and Singh H 1976 Preliminary studies on forecasting wheat stem rust appearance; *Agric. Meteorol.* **7** 281–289

Nagarajan S, Singh H and Joshi L M 1975 Climatic factors in relation to stem rust epidemiology; *Plant Dis. Reptr.* **59** 670–672

Nagarajan S, Singh H, Joshi L M and Saari E E 1976 Meteorological conditions associated with long distance dissemination and deposition of *Puccinia graminis tritici* uredospores in India; *Phytopathology* **66** 198–203

Nagarajan S, Singh H, Joshi L M and Saari E E 1977 Prediction of *Puccinia graminis* f. sp. *tritici* on wheat in India by trapping the uredospores in rain samples; *Phytoparasitica* **5** 104–108

Nagarajan S, Joshi L M, Srivastava K D and Singh D V 1978a Epidemiology of brown and yellow rusts of wheat in North India. III. Impact of varietal change; *Plant Dis. Reptr.* **62** 694–698

Nagarajan S, Joshi L M, Srivastava K D and Singh D V 1978b Synoptic meteorological conditions in relation to leaf rust spread in northern India; *Proc. First Intern. Conf. Aerobiology, held at Munich, West Germany,* August 1978 pp 446–451

Nagarajan S, Joshi L M, Srivastava K D and Singh D V 1979 Epidemiology of brown and yellow rusts of wheat in North India. IV. Disease management recommendations; *Cereal Rusts Bull.* **7** 15–20

Nagarajan S, Saari E E and Kranz J 1980 Conditions that led to the 1978 epidemic of *Puccinia striiformis* over North western parts of the Indian subcontinent—a preliminary report; *Proc. Vth European and Mediterranean Cereal Rusts Conf, Bari,* Italy, pp 213–216

Nagarajan S, Kranz S, Saari E E and Joshi L M 1982a Analysis of wheat rusts epidemics in the Indo-Gangetic plains 1976–78; *Indian Phytopath.* **35** 473–477

Nagarajan S, Selboldt G, Kranz J, Saari E E and Joshi L M 1982b Utility of weather satellites in monitoring cereal rust epidemics; *Pflkrankh* **89** 276–281

Nagarajan S, Bahadur P and Nayar S K 1984 Contemplating the management of brown rust resistance genes to mitigate the spread of *Puccinia recondita* f. sp. *tritici*; *Indian Phytopath.* **37** 490–497

Pal B P 1978 Wheat research—the early phase; *Indian Fmg* **27** 3–5

Pande S 1976 *Studies on the epidemiology of stem rust on wheat with special reference to sporulation of the pathogen* (*Puccinia graminis tritici*), Ph D Thesis, IARI, New Delhi 172 p

Pathak K D, Joshi L M and Chinnamani S 1979 Natural occurrence of *Puccinia graminis tritici* on *Brachypodium sylvaticum* during off season in Nilgiri hills; *Indian Phytopath.* **32** 308–309

Payak M M 1965 *Berberis* as the aecial host of *Puccinia brachypodii* in Simla hills (India); *Phytopath. Z.* **52** 49–54

Prasada R 1946 The uredostage of aecidium found on *Thalictrum* in Simla Hills; *Curr. Sci.* **15** 254–255

Prasada R 1947 Discovery of the uredo-stage connected with the aecidia so commonly found on species of *Berberis* in the Simla hills; *Indian J. agric. Sci.* **17** 137–151

Prasada R 1951 Rusts on wild grasses; *Curr. Sci.* **20** 243

Prasada R 1960 Fight the wheat rust; *Indian Phytopath.* **13** 1–5

Rajaram S, Bragcich P and Butler L 1984 Bread wheat improvement; *CIMMYT Report on Wheat Improvement* 1981 pp 1–15

Roelfs A P, Dirks V P and Romig R W 1968 A comparison of rod and slide samplers used in cereal rust epidemiology; *Phytopathology* **58** 1150–1154

Roelfs A P, Rowell J B and Romig R W 1970 Sampler for monitoring cereal rust uredospores in rain; *Phytopathology* **60** 187–188

Rowell J B, Olien C R and Wilcoxson R D 1958 Effect of certain environmental conditions on infection of wheat by *Puccinia graminis; Phytopathology* **48** 371–377

Sahni M L and Prasada R 1963 A study on the environmental conditions influencing the development of the three rusts of wheat in the neighbourhood of Delhi. Incidence of wheat rusts in relation to initial spore showers and weather condition; *Indian Phytopath.* **16** 285–294

Sharma S K, Singh S and Goel L B 1973 Note on a new record of Sonalika infecting race of yellow rust in India and sources of its resistance; *Indian J. agric. Sci.* **43** 964–965

Sharp E L, Schmitt C G, Staley J M and Kingsolver C H 1958 Some critical factors involved in establishment of *Puccinia graminis* var. *tritici; Phytopathology* **48** 469–474

Sleeman W H 1839 Extracts Major Sleeman's diary; *Trans. Agric. Hortc. Soc. India* **6** 79–87

Srivastava K D 1981 *Studies on the spread of Puccinia recondita Robs. ex. Desm. and its epidemiology*; Ph.D Thesis, Agra University, Agra

Srivastava K D, Joshi L M and Nagarajan S 1984a An assessment of leaf rust in multiline population of wheat; *Indian Phytopath.* **37** 306–311

Srivastava K D, Joshi L M and Singh D V 1984b Epidemic development of rusts in multiline population of wheat; In: *Progress in microbial ecology* (eds) K G Mukherjee, V P Agnihotri and R P Singh, Print House (India) Lucknow pp 179–193

Stamkan E C and Harrar J G 1957 *Principles of Plant Pathology*; (New York: Ronald Press Co) pp 581

Swaminathan M S 1978 Wheat revolution the next phase; *Indian Fmg* **27** 7–17

Swaminathan M S, Raychaudhuri S P, Joshi L M and Gera S D 1969 *Wheat Disease News Letter, IARI, New Delhi* **3** 1–8

Thorpe I G and Ogilvie L 1961 Black stem rust of wheat (*Puccinia graminis* var. *tritici* Pers.) Investigations in England 1955–60. *Second European Colloquium on black rust of cereals, Madrid*

Vasudeva R S, Joshi L M and Lele V C 1953 Susceptibility of some grasses to cereal rusts; *Indian Phytopath.* **6** 39–46

Zadoks J C 1959 On the formation of physiologic races in plant parasite; *Euphytica* **8** 104–116

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 407–413.
© Printed in India.

Identity of dactuliophora leaf spot of pearl millet

P D TYAGI

Department of Crop Protection, Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria

Abstract. The present investigation has shown that the leaf spot of pearl millet (Dactuliophora leaf spot) caused by *Dactuliophora elongata* has been erroneously named as zonate leaf spot and the causal agent misidentified as *Gloeocercospora* sp. by Williams *et al* (1978) and Sundaram (1980). The symptoms of Dactuliophora leaf spot are different from those of "true" zonate leaf spots of sorghum and pearl millet caused by *Gloeocercospora sorghi*. The zonate leaf spot pathogen, *G. sorghi*, produces few to many septate, hyaline, elongate to filiform conidia but no conidia are produced by *D. elongata*. In *G. sorghi*, sclerotia are produced sub-epidermally and germinate in 3–7 days by producing sporodochia. In *D. elongata*, sclerotia are produced on fully erumpent sclerotiophores and germinate in 4–8 hr by producing germ tubes all over the surface.

Keywords. Pearl millet; *Dactuliophora elongata*; dactuliophora leaf spot.

1. Introduction

In 1981 a foliage disease showing roughly concentric or zonate spots studded with black sclerotia was observed on many varieties/lines of pearl millet (*Pennisetum americanum* [L.] Leeke) at the experimental farm (field W_6) of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The disease assumed high severity by the middle of September resulting in premature drying up of leaves of susceptible varieties. The final symptoms of the disease resembled exactly those shown in a coloured photograph of a diseased pearl millet leaf on page 85 of the booklet "Sorghum and Pearl Millet Disease Identification Handbook" published by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad, India (Williams *et al* 1978). In this publication the disease is named zonate leaf spot and is said to be caused by *Gloeocercospora* sp. About the same disease, while working with ICRISAT at Zaria (Nigeria), Sundaram (1980) remarked, "It was interesting to observe the incidence of zonate leaf spot (*Gloeocercospora* sp.) in a severe form in field No. W_6". He further remarked, "It was surprising to note that most of the entries tested were susceptible to zonate leaf spot. The least infection in few entries observed was grade 3 (of 1–5 infection grades). Certain entries exhibited very severe premature drying up of the leaves due to zonate leaf spot infection". However, a detailed study of the development of symptoms and the fungus involved in the disease has shown that this leaf spot has been erroneously identified as zonate leaf spot by Williams *et al* (1978) and Sundaram (1980). It is identical with the leaf spot (*Dactuliophora elongata* Leakey) of pearl millet described by Leakey (1964) but is different from "true" zonate leaf spots (*Gloeocercospora sorghi* Bain and Edgerton ex Deighton) of sorghum and pearl millet first described by Bain and Edgerton (1943) and Luttrell (1954), respectively. An account of these studies and their bearing on correct identity of the disease and the pathogen is presented in this paper. Part of this work has

been published as an abstract wherein the disease was named "Dactuliophora leaf spot" to distinguish it from "true" zonate leaf spots of sorghum and pearl millet.

2. Materials and methods

2.1 *The pathogen*

2.1a *Isolation of the pathogen*: The diseased material was collected from the experimental farm (field W_6) of the Institute for Agricultural Research, Ahmadu Bello University, Zaria, Nigeria. The pathogen was isolated on potato dextrose agar with streptomycin (PDAS). Young lesions on leaves were cut out, surface-sterilized for 3 min in 1 % solution of sodium hypochlorite, and plated out on PDAS. Plates were incubated at room temperature (24–32°C).

2.1b *Morphology of the pathogen on host*: The morphological characters of the pathogen were studied using a hand-lens and a high-power microscope. Transverse and longitudinal sections and scrapings of the diseased leaves were also examined.

2.1c *Germination of sclerotia*: Sclerotia for germination were collected from mature lesions on pearl millet leaves. The glass slides containing the sclerotia in drops of water were maintained in moist chambers at room temperature (24–32°C). At 1 hr intervals after placement in water drops, the sclerotia were observed microscopically for germination.

2.2 *Pathogenicity tests*

Pathogenicity tests were done on 20-day-old healthy pearl millet plants of line KARS 5, grown in the glasshouse in sterilized potted soil (15 cm pots). The plants to be inoculated were first atomized with water. Sclerotia, collected from mature lesions on pearl millet leaves were applied to leaves with wet fingers by running the fingers gently over the surface in such a way as to ensure uniform distribution of inoculum. The plants were again atomized and kept in a saturated atmosphere for 72 hr. Control plants were kept isolated in identical conditions. After 72 hr the plants were removed from continuous humid atmosphere and during the next 3 days kept under an alternating cycle of 16 hr of humid atmosphere (1600–0800 hr) and 8 hr of sunlit open atmosphere (0800–1600 hr).

2.3 *Symptoms development under field conditions*

Isolated lesions in initial stages were marked on leaves of 10 plants of a highly susceptible pearl millet line (KARS 5). Growth of lesions was observed everyday for 30 days.

3. Results

3.1 *The pathogen*

The pathogen grew well on PDAS producing tan aerial mycelium but did not form sclerotia in culture.

On host the mycelium was generally immersed but white mycelium, very closely adpressed to the cuticle, was visible on leaf spots at the lower surface of the leaves. Mycelium was diffused throughout the spot and aggregated irregularly in the epidermal or deeper leaf tissues into plectenchymic masses from which sclerotiophores and sclerotia developed. Sclerotiophores were fully erumpent. The sclerotium was liberated from the living host by detachment from a cup-shaped sclerotiophore, which remained attached to the host and was visible as a ring of thick-walled, brown mycelium surrounding a circle of fractured cells from which the sclerotium had separated. Sclerotia were glabrous, oblate-ellipsoid to pyriform, 123–237 μ long and 98–188 μ broad at the widest point (figure 1).

The sclerotia germinated in 4–8 hr by producing mycelial germ tubes all over the surface.

3.2 *Pathogenicity tests*

The inoculated leaves showed minute lesions 4 days after inoculation. The lesions gradually grew in size and coalesced to form necrotic areas bearing sclerotia identical with *D. elongata*.

3.3 *Symptoms development under field conditions*

The disease started on the upper (adaxial) leaf surface by the appearance of pin-point, isolated brown lesions (figure 2a). These lesions gradually increased in size (figure 2b). They became brownish at the edge but dirty white or straw coloured towards the centre (figure 2c). In wet weather, irregular water-soaked areas developed around these spots (figures 2d–2f). On the lower surface, the water-soaked areas were covered by whitish

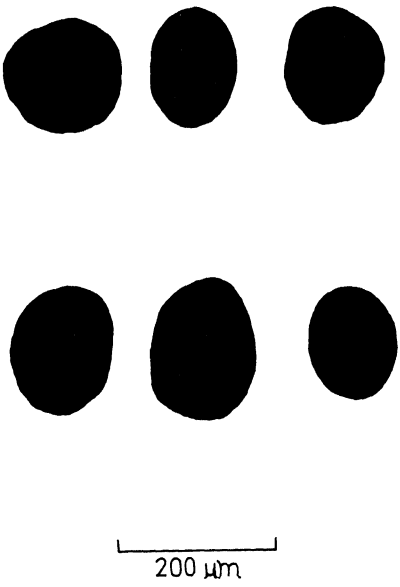


Figure 1. Sclerotia of *Dactuliophora elongata*.

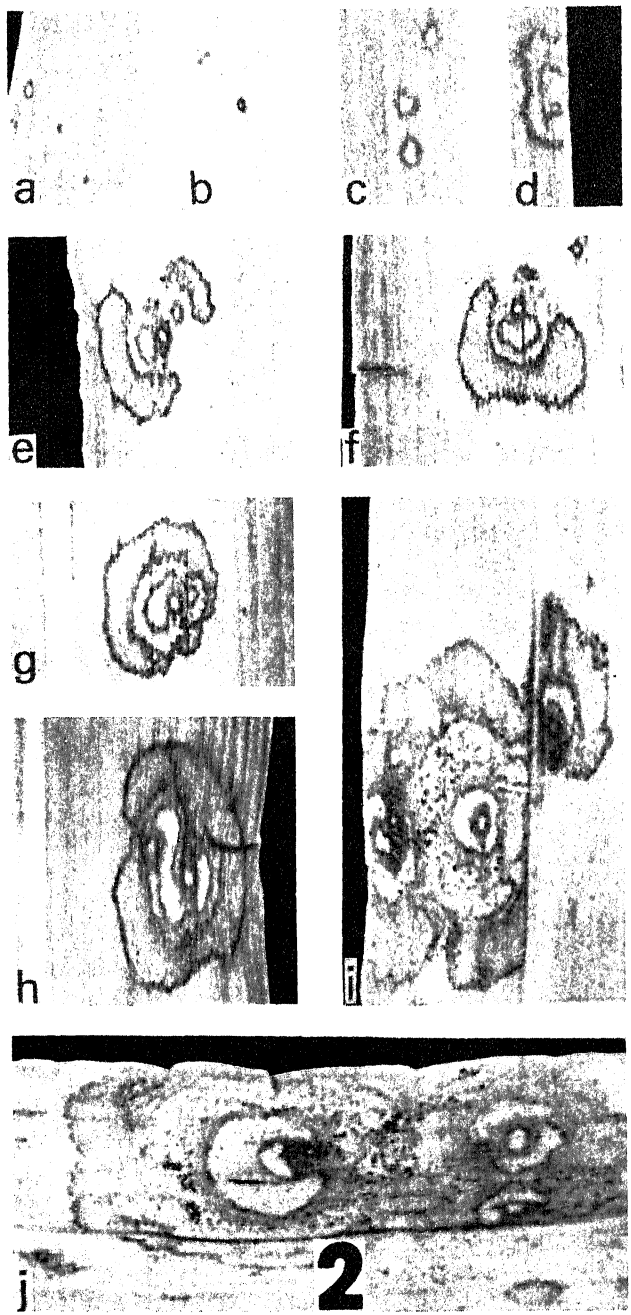


Figure 2(a–j). Different stages in the development of symptoms of Dactuliophora leaf spot.

mycelium of the fungus very closely adpressed to the host cuticle. After 3–4 days, the water-soaked areas became necrotic and assumed straw or brown colouration. These necrotic patches were then surrounded by fresh irregular water-soaked areas (figures 2g–2h) which were also covered by whitish mycelium on the lower surface of the leaves.

The newly formed water-soaked areas also became necrotic and this process (appearance of irregular water-soaked areas and their conversion into necrotic patches) continued till the lesions were fully formed (figures 2i–2j). The well developed lesions were either of no definite shape or roughly oval, circular or semi-circular, up to 10 cm long, zonated, with kidney-shaped patches of necrotic tissue and brown or yellowish areas in between. The shape and development of lesions slightly varied on different varieties/lines of pearl millet. The lesions in various stages of development on a leaf of cultivar ex-Borno are shown in figure 3. The necrotic lesions were studded with black sclerotia generally on the upper leaf surface although rarely they were also formed on the lower surface. In the final stages, the lesions coalesced to cover almost the entire leaf surface (figure 4) resulting in premature drying of leaves.

The final symptoms of Dactuliophora leaf spot (figure 4) resemble those of the so-called "zonate leaf spot" (*Gloeocercospora* sp.) on pearl millet illustrated by Williams *et al* (1978).

4. Discussion

This investigation has led the author to the conclusion that the leaf spot of pearl millet (Dactuliophora leaf spot) caused by *Dactuliophora elongata* Leakey is erroneously named as zonate leaf spot (*Gloeocercospora* sp.) by Williams *et al* (1978) and Sundaram (1980). Although *D. elongata* was first reported by Leakey on pearl millet as early as 1964, surprisingly it escaped the attention of pearl millet pathologists. Leakey (1964) briefly described the symptoms of the leaf spot from herbarium material deposited in Commonwealth Mycological Institute, London. However, no pathogenicity tests were attempted by him using *D. elongata*.

The concentric or zonate appearance of lesions in Dactuliophora leaf spot instantly recalls to mind the symptoms of zonate leaf spot of sorghum (Bain and Edgerton 1943) and of pearl millet (Luttrell 1954). This probably led Williams *et al* (1978) and Sundaram (1980) to regard the disease as zonate leaf spot. They, however, failed to recognize the following features which distinguish Dactuliophora leaf spot from "true" zonate leaf spot: Firstly, the development of symptoms of Dactuliophora leaf spot on pearl millet described in this paper is entirely different from those of zonate leaf spots of sorghum (Bain and Edgerton 1943) and of pearl millet (Luttrell 1954). Further, in zonate leaf spot of sorghum the initial lesions are reddish-brown and the mature ones show alternating bands of dark purple or red colour and tan or straw colour. In Dactuliophora leaf spot, lesions do not show purple or red colouration at any stage of development. Although Luttrell (1954) gave no illustration, the symptoms of zonate leaf spot of pearl millet described by him are different from those of Dactuliophora leaf spot.

Secondly, on sorghum under warm humid conditions, *G. sorghi* produces pinkish gelatinous fruiting bodies on and around the necrotic areas of the lesions (Bain and Edgerton 1943) while on pearl millet, tiny salmon-coloured globules are visible under the hand-lens on both surfaces of the spots (Luttrell 1954). In Dactuliophora leaf spot, no gelatinous fruiting bodies or salmon-coloured globules are produced on the lesions. The conidia of *G. sorghi* are few to many septate, hyaline, elongate to filiform. No conidia are produced by *D. elongata*.

Thirdly, the genesis and germination of sclerotia of *G. sorghi* and *D. elongata* are

Figures 3 and 4. **3.** Lesions of Dactuliophora leaf spot on a leaf of cultivar ex-Borno. **4.** The final stage of Dactuliophora leaf spot. Lesions have coalesced to cover the entire leaf surface.

markedly distinct. In *G. sorghi*, sclerotia are sub-epidermal and remain immersed in the mesophyll (Rawla 1973). They germinate after 3–7 days by producing sporodochia (Dean 1968). In *D. elongata*, the sclerotia are produced on fully erumpent sclerotiophores mainly on the upper leaf surface. They germinate after 4–8 hr by producing germ tubes all over the surface. Thus the method of sclerotial germination alone, which constitutes a very important taxonomic character, can distinguish *D. elongata* from *G. sorghi*.

References

Bain D C and Edgerton C W 1943 The zonate leaf spot, a new disease of sorghum; *Phytopathology* **33** 220–226

Dean J L 1968 Germination and overwintering of sclerotia of *Gloeocercospora sorghi*; *Phytopathology* **58** 113–114

Leakey C L A 1964 *Dactuliophora*, a new genus of Mycelia Sterilia from Tropical Africa; *Trans. Br. Mycol. Soc.* **47** 341–350

Luttrell E S 1954 Diseases of pearl millet in Georgia; *Plant Dis. Rep.* **38** 507–514

Rawla G S 1973 *Gloeocercospora* and *Ramulispora* in India; *Trans. Br. Mycol. Soc.* **60** 283–292

Sundaram N V 1980 Annual Report: UNDP/ICRISAT West Africa Cooperative Programme, Nigeria–1980. Part II. Cereal Pathology, 42 pp

Tyagi P D 1981 Dactuliophora leaf spot of pearl millet in Nigeria. Abstract. Third International Symposium on Plant Pathology, p. 27, 1981, New Delhi, India, 14–18 December 1981

Williams R J, Frederiksen R A and Girard J C 1978 *Sorghum and pearl millet disease identification handbook* Information Bulletin No. 2, ICRISAT, Hyderabad, India. 88 pp.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 415–431.
© Printed in India.

Management of *Rhizoctonia solani* diseases with chemicals

RAJENDRA K GROVER and HANS R KATARIA

Department of Plant Pathology, Haryana Agricultural University, Hissar 125 004, India

Abstract. Plant diseases caused by *Rhizoctonia solani* are generally managed by use of fungicides as seed and/or soil treatments. Disease control is reported to vary on different crops by the same chemicals on account of their use in different seasons and regions. Reasons for such a diversity in the efficacy of fungicides have been determined in some cases and these have been attributed to variations in soil characteristics like reaction, texture, moisture and cation exchange capacity, etc. In addition, the interaction of fungicides with fertilizers, manures, herbicides and insecticides applied to the crop have also been a cause of such a variation. The limitations of fungicides to control *R. solani* diseases satisfactorily under diverse crop habitats and their interactions with other agrochemicals have been discussed

Keywords. *Rhizoctonia solani*; seedling rot; fungicides; bio-ecological interactions.

1. Introduction

Rhizoctonia solani, first described by Kühn in 1858 on potatoes, occurs in all parts of the world both in cultivated and uncultivated areas. It is capable of attacking a wide variety of plant species causing seed decay, damping-off, root rots, stem rot and cankers, besides foliage and fruit diseases. Inspite of the fact that it has a very vigorous saprophytic ability to thrive in the soil, it also has lethal pathogenic potential on unlimited number of hosts. This has been a subject of hundreds of research papers, yet complete agreements on its taxonomy, biology, and several aspects of pathogenesis and control measures are wanting. Comprehensive reviews on *R. solani* as a plant pathogen are available (Duggar 1915; Boosalis 1950; Parmeter 1970; Anderson 1982).

2. General considerations

R. solani is a non-sporulating mycelial form having typical septation and branching with essentially multinucleate cells. Sclerotia are characteristic of the species which are mainly uniform in texture, varying in size from small, round often more than 1mm in diameter, to thin crusts, several centimeters across. It produces monilioid cells, often called barrel-shaped cells or chlamydospores, in chains or aggregate, sometimes referred to as sporodochia. Saksena and Vaartaja (1960, 1961) have placed considerable emphasis on the use of monilioid cells in distinguishing the species of *Rhizoctonia*.

Being heterokaryotic in nature, the variations in its morphology are numerous, often leading to confusion in its identification. Parmeter and Whitney (1970) have reviewed the taxonomy and nomenclature of *R. solani*, while that of its perfect state *Thanatephorus cucumeris* (Frank) Donk has been reviewed by Talbot (1970).

Variations in the virulence and host range of the field isolates of *R. solani* are many and these have been summarized by Kernkamp *et al* (1952), Richter and Schneider

(1953), Flentje and Saksena (1957) and Luttrell (1962). It has been amply demonstrated that representative field isolates of *R. solani*, while usually virulent on one or more hosts, may be avirulent, weakly virulent on a few hosts or highly virulent on a wide range of the host plants. Thus while pathogenicity to a wide range of plants is characteristic of *R. solani*, avirulence and restricted host range do not exclude isolates from *R. solani*. The extent of variation in cultural characteristics, saprophytic behaviour and virulence makes this pathogen a distinct entity which defies proper generalizations and management.

The colonization and saprophytic growth of *R. solani* in the soil have been summarized by Papavizas (1970). Sclerotia and resting mycelia are the chief means of survival in the soil, while its activity to colonize the substrates in the soil depends upon several micro-ecological conditions (Papavizas and Davey 1961; Martinson 1963; Weinhold 1977). Remarkable adaptation of *R. solani* to wide ranging characteristics of the soil and the related factors like microbial competition for nutrients, antagonism, hyper-parasitism etc., makes it a very versatile organism whose elimination from the infested soils becomes a difficult task.

In fact, the ubiquity and versatility of *R. solani* as a pathogen lies in its great potential of variability in nature, its capacity to parasitize a wide variety of host plants, and its great sustainance in the soil due to its enormous saprophytic ability. This unique pathogen, therefore, has been and is problematic on various cultivated crops often leading to visible damages of serious economic proportions. On the other hand, the absence of practical methods for natural repression of its population in the soil, absence of in-built resistance in the host plants, and its worldwide distribution on economic crop plants, have made it a suitable subject to be tackled by chemical means only (Leach and Garber 1970). Historically, the management of *R. solani* has been possible and achieved with the use of a variety of chemicals adopting diverse techniques. Yet, the success has been far from satisfactory in the fields, for the biology of the host:pathogen interactions in the realm of micro-environment of the soil is not understood in its totality. In this review, the control of *R. solani* by chemicals is presented with a special emphasis on the relationship of bioecological factors that influence the efficient control of this pathogen at large.

3. Use of chemicals against *R. solani*

Early work on the chemical control of *R. solani* was on potato black scurf. Steam-formalin treatment (Winston 1913), formaldehyde dip treatment (Chauzit 1923; Gilman and Melhus 1923) and hot formaldehyde at 50–55°C dip for 2–3 min (Raeder and Hungerford 1922; Rose 1925) were used with variable success. Mercuric chloride was tried by Quanjer (1916) and later by Dorst (1920) for disinfection of seed tubers. Rosen (1924) used the first organomercurial, uspulum (0·25 %) as soil drench for the control of seedling rot of cotton. Its dusting on potato tubers also gave good results (Lohman 1925).

With the development of organic fungicides, pentachloronitrobenzene (PCNB, quintozene) became very popular. Hooker (1935) used it as soil drench to control black scurf of potato and it has been subsequently used successfully as soil drench and as seed treatment to control *R. solani* on various crops. Last (1952) found that on lettuce 50 % dust of TCNB (tetrachloronitrobenzene) was as effective as 20 % PCNB dust. Captan, and

in some instances, zineb give good control when used as soil drench (Brinkerhoff *et al* 1954; Erwin *et al* 1957; Jhooty and Grover 1971). Quintozene in combination with other organic fungicides has also been tried with success. Recently, systemic fungicides, when used as soil drench or seed treatment, have provided excellent control of *R. solani* on many crop plants. Carboxin, chloroneb, ethazole, iprodione, and benzimidazole fungicides, *viz*, benomyl, carbendazim, thiophanate-methyl and thiabendazole, are highly effective.

Inspite of the fact that quite a large number of chemicals have been tried and found useful against *R. solani*, there appears to be a lack of agreement between different reports on the efficacy of a particular fungicide on a crop in different areas. In vitro tests conducted by various workers against different isolates of *R. solani* could not always be correlated with the field tests. The reasons for differential disease control with effective chemicals are many and are the subject of this review. The implications of such features, however, are yet to be realized for the effective field control of *R. solani* on all crops.

3.1 *Test methods and their implications*

Conventional test methods like seed treatment, soil drenching or dusting and fumigation have been employed for comparing the efficacy of different fungicides. In addition, a large number of workers have tested the sensitivity of *R. solani* to fungicides in nutrient media. Sinclair (1960) reported significant differences in the degree of sensitivity among five isolates and suggested that this may account for the apparent lack of uniformity of disease control in the field. Shatla and Sinclair (1962) found a correlation between pathogenicity and sensitivity of the isolates to quintozene while Maier (1962) reported that the differential in vitro sensitivity of 12 isolates to quintozene and thiram showed no correlation in the field tests. Jhooty and Bains (1973) also reported a lack of correlation between in vitro inhibition of growth of different *R. solani* isolates and disease control with fungicide treatments. Kataria and Grover (1978a) compared 41 fungicides in vitro against an isolate of *R. solani* and showed that these results could not be correlated in every case with the control of the pathogen on the host plant. Gapter (1968) demonstrated that *R. solani* isolates confined to surface form in the soil and susceptible to high concentrations of CO_2, were inhibited more by quintozene than the CO_2 resistant subterranean isolates.

It has also been shown by Kataria and Grover (1975a) that the fungicides preventing the development of infection cushions by *R. solani* on cotton threads and mung bean hypocotyls were those which also controlled the disease most efficiently. It has been contended that inhibition of such vulnerable structures, rather than the mycelial growth or their physiological characteristics, could be of advantage in correlating the laboratory evaluations with actual disease control tests.

3.2 *Field tests*

R. solani survives and multiplies in the soil and also on plant debris in the form of thick-walled hyphae and aggregation of monilioid cells forming loose sclerotia. The survival and multiplication of the fungus in the soil has been reviewed by Baker and Martinson (1970) with special reference to its epidemiology. (*R. solani* is also carried on and in the true seeds of many crop plants) (Baker 1947; Neergaard 1958). The mode of perpetuation of the pathogen necessitates the treatment of the soil and or the seed with

chemicals for overcoming the disease in germinating stages of the crop. No doubt, some cultural practices have been demonstrated to influence the population of *R. solani* in the soil, yet chemical control is of significance from the practical point of view. Moreover, *R. solani*, like many other soil-borne pathogens, is found in localized patches in the field where it causes recurring damages while seed-borne inoculum is responsible for widespread distribution and introduction of the pathogen in the fields. Chemical control, therefore, seems appropriate in such situations, and is briefly described below.

3.2a *Eradication and suppression of soil inoculum*: The primary consideration for the eradication of the soil-borne inoculum on a localized basis is to treat the portion of the demarcated soil by applying chemicals at such sites. Baker (1957) used dry or moist heat to eradicate *R. solani* from the soil of container-grown plants in greenhouses and nurseries. This method used in plant beds on small scale field plots has been both satisfactory as well as unsatisfactory. It is to be borne in mind that complete or near complete sterilization of the soil faces dangerous consequences if a pathogen is re-introduced into the soil with seed or by other means. In the absence of competition, *R. solani* grows luxuriantly and causes serious disease losses. Ferguson (1953) has shown that pepper plants sown in steam-sterilized soil are extensively damaged by *R. solani* because of seed contamination. But when selected antagonists are introduced into the soil immediately after sterilization, a healthy pepper crop is obtained. Several methods of heat sterilizing the soil have been tried by different workers, but this method has not come into general use for its obvious limitations (Leach and Garber 1970).

Fumigation of soil with general volatile toxic chemicals is rather expensive, and therefore it is mostly limited to greenhouse soils, small plant beds, nurseries and a few field grown crops that offer high financial returns. Most fumigants eliminate not only the pathogens but also the competitor-antagonistic organisms, and leave little or no residue because of their volatile nature. However, great care has to be exercised to prevent the re-introduction of the pathogen like *R. solani* through infested seed into the fumigated soil. Soil fumigation requires several considerations of the properties and functions of fumigants and their interactions with the soil components for their efficient use (Domsch 1964; Munnecke 1972).

Incorporation of solid fungicides in the form of granules or dust into large soil areas has also been attempted with a view to eliminate the resident *R. solani* inoculum. Livingston *et al* (1962) could control *Rhizoctonia* infections on potatoes by broadcasting quintozene which was disked into the soil surface layer. Thaxter (1890) was perhaps the first to advocate localized placement of fungicides in a seed furrow. He placed sulphur in the furrows to cotrol onion smut caused by *Urocystis cepulae* Frost. Seed furrow application of nabam has been found effective in controlling root rot complex which included *R. solani* on seedling beans and peas. With subsequent successes with furrow application of solid fungicides, a large number of chemicals were tested and found effective for the control of *R. solani* and other soil-borne pathogens. Localized placement of quintozene has proved to be of practical use and hence used each year over large areas to control *R. solani* on cotton in South America. Both granular form and dust have been employed on specially designed equipments. However, subsequent to continuous use of quintozene for controlling *R. solani*, the problem with other pathogens like *Pythium* spp. has been accentuated in South-West USA (Gibson *et al* 1961). Nevertheless, the practice of applying chemicals in the seed furrows has given

adequate control of *R. solani* in many crops, though the choice of chemicals, their selectivity and interaction with soil have led to newer problems yet to be resolved. In tropical countries, the use of quintozene as seed furrow treatment in cotton and a few other crops has been tried on a limited scale with erratic success. Reasons for this are yet eluding.

3.2b *Elimination of seed-borne inoculum*: Soil treatment with chemicals is also effective in eliminating externally seed-borne inoculum of *R. solani* where the chemical comes in direct contact with the germinating seed. Treating the seed with chemicals is commonly practised because of its advantages in protecting the seedlings at initial stages both from the seed- and soil-borne inoculum. However, when the inoculum density is high in the soil, the seed treatment may not be effective to that extent, as demonstrated by Benson and Baker (1974). In a series of experiments, Leach *et al* (1959, 1960) found that post-emergence damping-off of several crops was reduced when seeds were treated with quintozene or other effective fungicides, such as mercury compounds, thiram, captan etc. Kataria (1973) has compiled the literature on the control of *R. solani*, affecting various crops, by the use of seed treating chemicals. The most successful and widely used seed dressing fungicides reported are: quintozene, carboxin, carbendazim, benomyl, chloroneb and thiabendazole.

In a detailed study where 17 fungicides were used as seed treatment for controlling damping-off of mung bean due to *R. solani*, thiabendazole, carbendazim, benomyl, thiophanate-methyl, chloroneb, dichlozoline and dimethachlor were found superior to several other recommended fungicides like carboxin, quintozene and organo-mercurials (Kataria and Grover 1978a). The disease controlling performance of these fungicides could not, however, be related to their in vitro toxicity to fungus growth. Diverse results with action of fungicides against *R. solani* in vitro and in vivo have been reported by several workers but no reasons have been advanced as to the failure of the recommended fungicides to control the pathogen in different regions on the same or different crop.

In recent years, some efforts have been made to look into the interactions of seed treating fungicides in relation to soil characteristics, host genera, species and cultivars, other agrochemical inputs in field and the pathogen itself. Kataria and Grover (1976c) have described that the performance of fungicides to control *R. solani* varied on account of soil factors like temperature, pH, moisture, and texture, besides the host genera. Literature reveals that there are several interactions that influence the triangular interaction of host, pathogen and fungicides which may lead to variations in results of disease control. These are briefly reviewed.

4. Factors affecting efficacy of fungicides

4.1 *Soil factors*

4.1a *Texture*: The activity of fungicides in soil is determined by their availability in free and stable form. Their availability is determined by soil texture, particularly the clay minerals, organic matter content and cation exchange capacity (CEC) (Domsch 1964). Rushdi and Jeffers (1956) noted that the differential adsorptive capacity of soils owing to clay content, organic matter and CEC was responsible for variable disease

development and fungicidal control. Activity of fungicides was relatively poor in loam and clay loam soils. Kataria and Grover (1976c) found that benomyl, thiophanate-methyl, chloroneb and quintozene were relatively more effective in sandy loam than in loam or clay loam soils. Subsequently, Hans *et al* (1981) have reported that carbendazim seed treatment was effective at low concentrations in sandy soil, while in clay loam and sandy loam higher concentrations were required for similar control of the disease. These results were correlated with the different characteristics and adsorptive capacities of the soils in which the proportions of silt, clay, organic matter and CEC varied. Adsorption studies conducted with carbendazim and thiabendazole have shown that the availability of the fungicides in the soil is inversely proportional to clay minerals and organic matter content (Aharonson and Kafkafi 1975a, b). Owing to free charges on their surface, clays and organic matter components tightly bind the fungicides on account of strong adsorptive forces. The significance of adsorption lies in the distribution and availability of the fungicides in and around the root system for protection againt soil-borne pathogens. Khan (1980) reviewed the role of clay minerals and organic matter in soil in influencing the performance of weedicides and insecticides, but little is known about fungicides.

In a recent study by Sunder (1982), the interaction of two clay minerals, *viz*, montmorillonite and kaolinite has been investigated in relation to the disease controlling efficacy of fungicides against *R. solani*. He found that the efficacy of six systemic and three non-systemic fungicides decreased when montmorillonite or kaolinite were added to the sandy soil. Efficacy of captafol, MEMC, carbendazim, benomyl and thiophanate-methyl was most severely reduced by both the clays while kaolinite had no significant effect on the efficacy of carboxin, chloroneb and thiabendazole. Montmorillonite clay has higher CEC and more reactive surface area than kaolinite and thus the former could sequester greater amounts of fungicides introduced in the soil on the seed and therefore reduced the disease control more significantly. Kaolinite, on the other hand, can adsorb lesser amount of fungicides owing to its lower CEC and consequently results in lesser inactivation. However, a positive correlation of molecular structure of fungicides and adsorption on the two clays could not be established. Bailey *et al* (1968) reported that hydrophobic pesticides of low water solubility are more adsorbed on the clays. Seed treating fungicides, which by and large have very low solubility in water, are more vulnerable to adsorption by clays, resulting in decreased efficacy in disease control. Since little is known about the charge characteristics and ionization pattern of fungicides, it is not possible to advance reasoning for differential inactivation of fungicides by clay minerals. At the same time, however, it can be said that clay contents of the soils decidedly interact with the fungicidal performance in controlling diseases in the field.

4.1b *Soil pH*: *R. solani* grows over a wide range of pH (2–11), with optimum pH for different isolates ranging from 5·6 to 8·5 (Parmeter 1970). In the soil this pathogen causes the disease over the pH ranges that are good for plant growth. However, several workers (Frederiksen *et al* 1938; Vasudeva and Ashraf 1939; Bloom and Couch 1958; Bateman 1962,1963) failed to find any relation of the pH of soil with disease severity. On the other hand, Jackson (1940), Roth and Riker (1943) and Lewis and Papavizas (1977) reported different pH optima for disease development. Tisdale and Nelson (1960) believe that soil reaction influences the availability of certain ions which in turn have been linked with disease development. In North India neutral to slightly alkaline

soils have been found to favour *R. solani* diseases in different crops.

Rarely the soil pH has been taken into account while using fungicides to control *R. solani*. The studies on in vitro toxicity of fungicides against *R. solani* in substrate media of different pH have shown that, irrespective of optimum pH for growth of the organism, each fungicide has a specific pH range at which maximum antifungal activity is exerted (Adams *et al* 1971; Grover and Chopra 1972; Malhan *et al* 1975; Kataria and Grover 1976c; Kataria *et al* 1976; Hans *et al* 1981). Extending these studies further it was seen that in soils of variable pH, the efficacy of the fungicides in controlling *R. solani* on different hosts could be related to the in vitro pH optima for the toxicity of fungicides. For benzimidazole fungicides, neutral to alkaline soils are conducive for optimum disease control, while chloroneb and quintozene are more effective in acidic soils (Kataria and Grover 1976c; Hans *et al* 1981).

Such interactions of soil pH on the disease controlling potential of fungicides cannot be easily explained since the soil reaction is a complex ultimate of various biological and ecological interactions. However, there is an evidence that fungicides like thiram are rapidly decomposed in acidic soils, thus leading to the impairment in their efficacy (Kluge 1969). Protonation of carbendazim molecules and their adsorption on clay particle in soils of lower pH have been reported (Aharonson and Kafkafi 1975a). Coursen and Sisler (1960) have pointed out that substrate pH modifies the toxicity of fungicides by affecting the action of specific enzymes and influences the cell wall permeability. Greater inhibition of cellular processes in yeast has been observed because of increase in permeation of carbendazim into the cells in alkaline pH range (Hammerschlag and Sisler 1972). It is thus obvious that substrate pH has a specific role to play in the reaction of fungicides with the pathogen in the soil and the immobilization of the latter is related to the extent of decomposition of the fungicide at a specific pH, ability of the fungicide to permeate into the fungus cells or the dearrangement of metabolic processes, reactions and interactions.

4.1c *Soil temperature*: The ability of *R. solani* to grow from 2 to 40°C with an optimum ranging from 18 to 30° of different isolates is indicative of its remarkable adaptability (Parmeter 1970). Earlier work of Balls (1906) and Richards (1921,1923) showed that disease severity is merely not a function of fungal growth. In different crops, different optima for disease severity have been recorded (Baker and Martinson 1970), and this bears testimony to the conjuncture of Balls, and Richards. In general, for winter crops a temperature of 18 to 22° is optimum for *R. solani* disease development, and 26 to 32° is optimum for summer crops.

For the control of *R. solani* in both the seasons, the optimum temperature reactive range of the fungicides assumes significance, particularly in the tropical regions where the temperature fluctuations from season to season and day and night are wide. Benzimidazole fungicides, for instance, are effective as seed treatment at low temperatures (18–22°C) against *R. solani* infections, while chloroneb and quintozene are most reactive at 28–32°C (Kataria and Grover 1976c). Blaszczak *et al* (1977) found that seed tuber treatment with MEMC, and rhizocton controlled *R. solani* infection of potato sprouts most efficiently at 10°C but the disease control was relatively poor at 15° and 17·5°. Hans *et al* (1981) and Grover and Thareja (1983) have worked out the reactive range of carbendazim for controlling root and foliage pathogens, respectively, and showed that the fungicide was most effective at 20°C in both cases, irrespective of the pathogen or the host. Such a specificity of temperature required for the optimal

disease controlling ability of fungicides is of great practical significance, specially in the tropics and needs to be studied in greater details.

4.1d *Soil moisture*: As early as 1904 Rolfs (1904) found that poorly drained soils favour the development of *R. solani* on potatoes. Heavy irrigation and water logging condition predispose many crop plants for severe disease development (Schaal 1935; Menon *et al* 1952; Papavizas and Davey 1961). *R. solani* grows optimally at 100% rh and its growth is retarded at 99·5% rh (Schneider 1953). Schmiederknecht (1960) designated *R. solani* as a hygrophyle having a straight line or a concave growth curve with an optimum at 100% humidity. Lewis and Papavizas (1977) have experimentally supported the assumption that increased humidity leads to increase in severity of diseases caused by *R. solani*.

It is well-known that most fungicides act best in liquid state, though most of the solid fungicides are little soluble in water. Liquid state of fungicides rarely exists in the fields. Usually during the plant growth variable levels of water stress are experienced by the plant and the stress quantum is of particular significance when fungicide-treated seeds are in the process of germination. While moisture favours germination, its stress influences the fungicidal interactions with the pathogen. Crossan (1965) showed a relationship between the amount of soil moisture and the efficacy of quintozene against *R. solani*. Efficacy of quintozene is markedly reduced if the irrigation interval during germination in pot tests is increased from 24 to 72 hr, while such is not the case with benomyl and thiophanate-methyl to that extent (Kataria and Grover 1976c). Hans *et al* (1981) demonstrated that carbendazim seed treatment (at 1 g/kg) gave nearly complete disease control in adequately moist soil irrigated every 24 hr but failed to provide any control of the disease in relatively drier soil irrigated every 72 hr; in the latter soil increased dosage of carbendazim on the seed did not compensate the loss in efficacy due to moisture stress. Increase in the water content of the soil is known to enhance the mobility of several fungicides including systemics, because of rapid desorption from soil particles, thus leading to better disease control (Munnecke 1972). Conversely, the efficacy of fungicides is generally low as the water stress increases. Possible reasons for the loss in the disease controlling activity of fungicides in relatively dry or rainfed soils may include volatilization, thermal inactivation and lower desorption. Lack of good control of root pathogens by fungicides in tropical rainfed soils can thus be explained on this basis.

4.2 *Fertilizers and manures*

Agricultural soils are applied with inorganic and organic fertilizers, including green manures and micronutrients, so as to obtain optimum crop growth and yield. Such fertilizer-amended soils also have great impact on the growth, and saprophytic colonization of the substrates by *R. solani* in the soil. Papavizas (1970) has reviewed the effect of soil fertilization on substrate colonization, saprophytic activity and pathogenesis of *R. solani* and pointed out that the organic amendments influence the C:N ratio and most suppress *R. solani* activities in the soil by promoting the competitive organisms. Several experiments, on the other hand, have shown that potassium, nitrogen, or calcium deficiencies increase the disease potential of *R. solani*. Disease potential also increases with excessive nitrogen fertilization. Albrecht and Jenny (1931), Hynes (1937), Zaleski and Blaszczak (1954) and others have shown that

damping-off and seedling rot of the crops are high when calcium supply to the soil is less. Hynes (1937) controlled root rot of wheat with the addition of ammonium sulphate, while Janssen (1929) controlled *Rhizoctonia* disease of potato and Small (1927) controlled *R. solani* foot rot of tomato with this fertilizer. High salt concentrations, however, predispose the plants to infection by *R. solani* (Beach 1949). Singh and Singh (1957) found that incorporation of well-decomposed farmyard manure resulted in less root rot and less killing of cluster bean plants in fields. They attributed the success of disease control with farmyard manure to increased plant vigour and enhanced tissue maturity resulting from better plant nutrition. Thus it appears that incorporation of any organic manure or plant residue into the soil affects the nutrient status of the soil both for the host and the pathogen, along with that of the numerous other soil microbes. The impact of such soil-amendments on the efficiency of disease control with fungicides also becomes important since the physiology of interaction of host and pathogen is modified differentially with different nutrients incorporated into the soil.

Recently some evidence has been gathered to show that fertilizers, applied to the soil, markedly influence the efficacy of fungicides used for controlling *R. solani* diseases. Kataria *et al* (1981) found that N, P, K fertilizers and micro-nutrients influenced the efficiency of MEMC in controlling *R. solani* seedling rot of cowpea. Efficacy of quintozene was relatively less affected by these amendments, while carbendazim retained its efficacy without any loss. Among macronutrients, nitrate form of N was more reactive than amide and ammoniacal nitrogen in defying disease control by the three fungicides. Amide and ammoniacal nitrogen fertilizers are quickly adsorbed on soil colloids (Pesek 1964; Sauchelli 1964; Stevenson 1964; Thompson and Troeh 1979). The reactivity of ammoniacal nitrogen is reduced owing to its rapid volatilization in alkaline sandy soils of low cation exchange capacity (Mills *et al* 1974; Fenn and Kissel 1976; Thompson and Troeh 1979).

Phosphatic fertilizers, relatively immobile in soil, exert differential effects on different fungicides. Efficacy of carbendazim against *R. solani* is improved by both monocalcium phosphate and potassium dihydrogen phosphate, but these two phosphatic fertilizers exhibit differential behaviour towards MEMC and quintozene (Kataria *et al* 1981). Whether this differential action is due to phosphorus or the other constituents of the phosphatic fertilizers, *viz*, Ca and K needs further experimentation. Addition of potassium salts lowers the micro-pH of the soil (Teuscher and Adler 1960) and the fungicidal activity in soil is influenced by pH (Kataria and Grover 1976c; Hans *et al* 1981): this might explain the respective potentiation or deterioration of the activity of quintozene and carbendazim seed treatments in potassium-amended soils.

Another observation made by Kataria *et al* (1981) was that fertilization of the soil with N, P and K (100 : 20 : 60 μg/g soil) led to betterment in disease control by MEMC and quintozene, in contrast to the results when these fertilizers were used singly. Sunder (1982), however, found that N, P and K added into the soil in the ratio 50 : 50 : 63 μg/g severely reduced disease control by MEMC, quintozene, carbendazim, benomyl and thiophanate-methyl. Obviously the relative proportion of these fertilizers in the soil determines their interactive modes with fungicides.

The effects of soil amendments with organic manures on the fungicidal control of *R. solani* affecting cotton and cowpea have been investigated by Bandyopadhyay *et al* (1982) and Sunder (1982). They found that biogas sludge and farmyard manure annulled the efficacy of MEMC and quintozene and also markedly reduced the efficacy of carbendazim. The magnitude of reduction in fungicidal activity varied with the test

fungicides, the type and quantity of manure and the host species. Green manuring with *Sesbania aculeata* ('daincha') also affected the disease control but the effects were far less than those of biogas sludge and farmyard manure. This is perhaps because the amendments with *S. aculeata*, like other green manure crops, add less organic matter to the soil than is provided by decomposed organic manures (Teuscher and Adler 1960). Bandyopadhyay *et al* (1982) and Sunder (1982) attributed the inactivation of fungicides by organic manures to humic acid present in these manures. Humic substances are colloidal in nature and possess a very high CEC (Schnitzer and Khan 1972), and the fungicides are likely to be adsorbed on the humus in the soil (Aharonson and Solel 1976; Frissel and Poelstra 1979) and rendered inactive against the pathogen. All fungicides, however, do not interact with organic manures and humic substances in the same way. For instance, carboxin, and also chloroneb to a lesser extent, resist inactivation by humic acid while MEMC, quintozene, captafol, and the benzimidazole fungicides lose their efficacy in soil amended with humic acid (Sunder 1982). Moreover, the water extract of organic manures improves the efficiency of quintozene, though this fungicide is inactivated in soil amended with biogas sludge. Conversely, activity of carboxin is reduced by the same water extract, though the intact manure has little effect on the efficacy of this fungicide. This implies that it is not only the humic acid which is responsible for reducing the efficiency of seed treating fungicides, but also the water soluble components (including fulvic acid) which inactivate the fungicides like carboxin and chloroneb. The precise reaction of the complex organic manures with the activity of seed treating fungicides is yet to be worked out.

Micronutrients which play a vital role in improving crop production, also react with fungicides and influence their disease controlling efficiency. Available forms of Zn, Fe and Cu inactivate MEMC and quintozene but not carbendazim used as seed treatment to control *R. solani* on cowpea (Kataria *et al* 1981). In a detailed study with six micronutrients added in the soil and nine fungicides used as seed treatment, Kataria and Sunder (1983) found that Fe^{++} reduced the efficacy of MEMC, quintozene, captafol, thiophanate-methyl, chloroneb and carboxin against seedling rot caused by *R. solani*; Cu^{++} and Zn^{++} annulled the activity of captafol, quintozene, thiabendazole and thiophanate-methyl, and Mn^{++} decreased disease control by captafol and thiophanate-methyl. None of the six micronutrients lowered the efficacy of carbendazim. Reversal of the toxicity of fungicides in the presence of metal ions has been attributed by Zentmyer (1944) to the formation of metal complexes, and or their metal chelating ability (Horsfall 1956; Lukens 1971). Fe salts reversed the toxicity of benomyl, thiophanate-methyl and chloroneb to *R. solani* (Kataria and Grover 1978b); it was speculated that these fungicides would not be effective in Fe-rich soils. Manji *et al* (1971) have shown less fungitoxicity to *Monilinia laxa* of the mixture of benomyl + copper (Bordeaux mixture) than benomyl alone in the field. Tests in UK have also revealed that thiophanate-methyl is not compatible with copper fungicides (Worthing 1979).

The fact that carbendazim remains effective in presence of metal ions, is probably because its primary mode of action is on DNA synthesis (Clemons and Sisler 1971) and not on the metal dependent electron transport, and cellular permeability as is the case with benomyl and quintozene, respectively (Kataria and Grover 1975b, 1976a). Carboxin, chloroneb, thiabendazole and thiophanate-methyl, which are severely inactivated by metal ions, are known to damage mitochondrial activities and to create a blockage of metal dependent electron transport chain (Allen and Gottlieb 1970;

Ragsdale and Sisler 1970; Kataria and Grover 1976a; Lyr and Werner 1982).

Boron, oxygenated as in Borax, is unique in being quite inert and incapable to form complexes with inorganic and organic molecules (Cotton and Wilkinson 1980). Boron and molybdenum are of special interest since their presence improves disease control by MEMC, quintozene, chloroneb and carboxin (in case of boron) and carbendazim (in case of molybdenum) (Kataria and Sunder 1983). The importance of micronutrients in modifying the disease control potential of seed treating fungicides, therefore, assumes a special significance since wider application of these elements is being advocated and practised in intensively cropped agricultural soils. The diversity in results for disease control by fungicides, would then have to be viewed in this context as well.

4.3 *Other agrochemicals*

Application of herbicides to soils is becoming a routine in crop production. The impact of these chemicals on root diseases and soil-borne pathogens has already been reviewed (Altman and Campbell 1977, 1979). No generalizations have so far emerged out of the studies on the effect of soil applied herbicides on the disease development, as the studies conducted so far are very few and in limited areas with few chemicals only. Moreover, the effects of herbicides on root diseases are likely to vary with variations in climatic conditions in different seasons and regions (Wyse *et al* 1976; Kataria and Dodan 1981, 1983).

Since herbicides are lodged in the soil where also is the fungicide and the pathogen along with the growing host roots, it is pertinent to assume that a possible interaction may occur between the two groups of chemicals in the soil environment. Pinckard and Standifer (1966) observed that infection of cotton by *R. solani* was increased by incorporation of the herbicide trifluralin in soil, but was almost eliminated when quintozene was used in combination with the herbicide. Kataria and Dodan (1982) found that pre-planting soil herbicides fluchloralin and alachlor modified the disease controlling ability of quintozene and carbendazim used as seed and soil treatments for cowpea against *R. solani*. Quintozene gave better disease control in soil pre-treated with either of the two herbicides, while disease control by carbendazim was decreased in such treated soil. Further studies are needed to elaborate on the interaction of herbicides, insecticides and other agrochemicals with fungicides used for the control of soil-borne pathogens.

4.4 *Host factors*

R. solani has a very wide host range and is distributed in a variety of agroclimatic and soil zones of the world. Strain differences as well as narrow host range specificity have also been recorded (Baker 1970). As stated earlier, a large number of fungicides have been found effective to control this pathogen on different crops, but the efficacy of the recommended fungicides has not been found to be equally good and uniform on various crops. Kataria and Grover (1976c) found distinct differences between the disease controlling potential of thiophanate-methyl, benomyl, chloroneb and quintozene tested on four hosts belonging to different genera. Comparatively, benomyl seed treatments gave satisfactory control of *R. solani* on mung bean, egg plant, and sugarbeet but not on long melon, while chloroneb gave best performance on mung bean and sugarbeet but poor control on eggplant and long melon, etc. This study conducted under uniform environmental conditions and with a single *R. solani* strain

strongly suggests that the hosts have a positive contribution in mediating the effects of fungicides in the disease control process. Sommartaya (1980) extended this study to several pathogens, including *R. solani*, and several host genera, species and cultivars, and found that the host's exudates participated in a positive manner in altering the antifungal activity of different fungicides. Reactive chemical components of root or foliage exudates were fractioned and their role in altering the toxicity of the fungicides was confirmed. These studies highlight the importance of the host, its cultivar and species in conducting disease control tests with chemicals and question the assumption that the pathogen targetted effective fungicides would be equally good on all susceptible host genera, species and cultivars.

5. Futuristic approach

Rhizoctonia solani, a pathogen of great diversity, preponderance and having wide adaptability to diverse soils and plants, is hard to reckon with, for genetic resistance in hosts is scarce, while other methods for its management are fraught with complications and over-ridden with biologically complex interactions. Although a variety of chemicals are known to control *R. solani* attacking different crops in different areas, yet failures to control the disease on the same crop in another zone are equally well documented in literature. Diversities in results for satisfactory disease control have hardly been reasoned scientifically. The purity of the fungicides, their formulations and the improper usage have often been the alibi for the extension workers to console the cultivators for poor disease control with the tested anti-*R. solani* fungicides. The information so far available discount this contention.

R. solani is a soil- and also seed-borne pathogen and its survival and multiplication in soil and the pathogenesis on the numerous hosts is governed not only by the susceptibility of the host it attacks, but also on the micro-environment around the site of interaction. Besides, it is now being known that the fungicidal chemicals also have a specific reactive range of activity which is greatly influenced by the soil environment and the associated agronomic factors. Under such a situation, the action of the recommended fungicides to control *R. solani* on a given host is likely to vary and it has. But studies on quadrangular interaction of host:pathogen:environment:fungicide have been rather few and as a consequence, erratic control of the pathogen on different crops has been the dividend.

The empirical reasons for the ineffectiveness of the chemicals to control *R. solani* in the fields have been highlighted. Apparently, the reasons for the failures of the chemicals to control the pathogen are many and intricate, yet the information so far gathered also demonstrates that there are some chemicals which are effective under a wide range of micro-environmental conditions and could give satisfactory disease control under most situations. However, the need to understand the reasons for our poor knowledge about the fungicidal interactions in the inter-play of host: pathogen: environment, assumes special significance since it is contrary to our belief that a potent antifungal chemical shall curb the pathogen under all conditions. Future studies have to be diverted for better understanding of the fungicidal behaviour as influenced by environment.

References

Adams P B, Warren H L and Tate C J 1971 Potassium azide: fungicidal activity as affected by pH; *Plant Dis. Rep.* **55** 1077–1081

Aharonson N and Kafkafi U 1975a Adsorption of benzimidazole fungicides on montmorillonite and kaolinite clay surfaces; *J. Agric Fd. Chem.* **23** 434–437

Aharonson N and Kafkafi U 1975b Adsorption, mobility, and persistence of thiabendazole and methyl 2-benzimidazolecarbamate in soils; *J. Agric Fd. Chem.* **23** 720–724

Aharonson N and Solel Z 1976 Accumulation pattern and fungicidal effect of carbendazim and thiabendazole in peanut plants after soil injection; *Pestic. Sci.* **7** 441–446

Albrecht W A and Jenny H 1931 Available soil calcium in relation to "damping-off" of soybean seedlings; *Bot. Gaz.* **92** 263–278

Allen P M and Gottlieb D 1970 Mechanism of action of the fungicide thiabendazole, 2-(4-thiazolyl) benzimidazole; *Appl. Microbiol.* **20** 919–926

Altman J and Campbell C L 1977 Effect of herbicides on plant diseases; *Ann. Rev. Phytopathol.* **15** 361–385

Altman J and Campbell C L 1979 Herbizide and Umwelt: eine Ubersicht uber fordernde und hemmende Interaktionen mit Pflanzenkrankheiten; *Z. PflKrankh. PflSchutz.* **86** 280–302

Anderson N A 1982 The genetics and pathology of *Rhizoctonia solani*; *Ann. Rev. Phytopathol.* **20** 329–347

Bailey G M, White J L and Rothberg T 1968 Adsorption of organic herbicides by montmorillonite: Role of pH and chemical characters of adsorbate; *Soil Sci. Soc. Am. Proc.* **32** 222–234

Baker K F 1947 Seed transmission of *Rhizoctonia solani* in relation to control of seedling damping-off; *Phytopathology* **37** 912–924

Baker K F 1957 Damping-off and related diseases; *California Agric. Exp. Sta. Manual* **23** 34–51

Baker K F 1970 Types of *Rhizoctonia* diseases and their occurrence. In *Rhizoctonia solani, biology and pathology* (ed.) J R Parmeter Jr (Berkeley, Los Angeles and London: University of California Press) 125–148

Baker R and Martinson C A 1970 Epidemiology of diseases caused by *Rhizoctonia solani*. In *Rhizoctonia solani, biology and pathology* (ed.) J R Parmeter Jr (Berkeley, Los Angeles and London: University of California Press) 172–188

Balls W L 1906 Physiology of simple parasite. *Khediv. Agric. Soc. Yearbook* (Cairo) 173–195

Bandyopadhyay R, Yadav J P S, Kataria H R and Grover R K 1982 Fungicidal control of *Rhizoctonia solani* in soil amended with organic manures; *Ann. Appl. Biol.* **101** 251–259

Bateman D F 1962 Relation of soil pH to development of Poinsettia root rots; *Phytopathology* **52** 559–566

Bateman D F 1963 Factorial analysis of environment and pathogens in relation to development of the Poinsettia root rot complex; *Phytopathology* **53** 509–516

Beach W S 1949 The effects of excess solutes, temperature and moisture upon damping-off; *Pennsylvania Agric Exp. Sta. Bull.* **509** 29 pp.

Benson D M and Baker R 1974 Epidemiology of *Rhizoctonia solani* pre-emergence damping-off of radish: Inoculum potential and disease potential interaction; *Phytopathology* **64** 957–962

Blaszczak W, Weber Z and Manka M 1977 The effect of temperature upon the pathogenicity and chemical control of *Rhizoctonia solani* K. on potato tubers (*Solanum tuberosum* L.); *Acta Agrobot.* **30** 159–165

Blaszczak W, Weber Z, Glebezynski E and Kulczak L 1978 Zwalczanie rizoktoniozy ziemniaka (*Rhizoctonia solani* K.) przez zaprawianie sadzeniakow; *Rocz. Nauk Roln.* **8** 101–114

Bloom J R and Couch H B 1958 Influence of pH, nutrition, and soil moisture on the development of large brown patch; *Phytopathology* **48** 260

Boosalis M G 1950 Studies on the parasitism of *Rhizoctonia solani* Kuhn on soybeans; *Phytopathology* **40** 820–831

Brinkerhoff L A, Oswalt E S and Tomlinson J F 1954 Field tests with chemicals for the control of *Rhizoctonia* and other pathogens of cotton seedlings; *Plant Dis. Rep.* **38** 467–475

Campbell L 1954 An economic control of black root of cauliflower; *Plant Dis. Rep.* **38** 859

Chauzit J 1923 Potato diseases: methods of control; *Prog. Agric. Vitic.* **28** 13–21

Clemons G P and Sisler H D 1971 Localization of the site of action of a fungitoxic benomyl derivative; *Pestic. Biochem. Physiol.* **1** 32–43

Cotton F A and Wilkinson G 1980 Advanced inorganic chemistry 4th edn (New York and Toronto: John Wiley and Sons)

Crosier W F, Nash G T and Crosier D C 1970 Differential reaction of *Pythium* sp. and five isolates of *Rhizoctonia solani* to fungicides on pea seeds; *Plant Dis. Rep.* **54** 349–352

Crossan D F 1965 Field and greenhouse experiments for control of *Rhizoctonia* root rot of snapbean; *Phytopathology* **55** 503

Davis J R and Callihan R H 1970 Evaluation of several fungicides in Idaho for control of *Rhizoctonia* on potato; *Phytopathology* **60** 1533

Domsch K H 1964 Soil fungicides *Ann. Rev. Phytopathol.* **2** 293–320

Dorst J C 1920 Sublimaat behandeling van Pootaardappelen; *Freisch. Landb.* **21** 47–48

Duggar B M 1915 *Rhizoctonia crocorum* (Pers.) DC. and *R. solani* Kuhn (*Corticium vagum* B. & C.) with notes on other species; *Ann. Missouri Bot. Garden* **2** 403–458

Erwin D C, Sappenfield W P and Kortsen R 1957 Effect of some fungicides on seedling diseases of cotton in the irrigated desert valleys of southern California; *Plant Dis. Rep.* **41** 324–329

Fenn L B and Kissel D E 1976 The influence of cation exchange capacity and depth of incorporation on ammonia volatilization from ammonia compounds applied to calcareous soils; *Soil Sci. Soc. Am. Proc.* **40** 394–398

Ferguson J 1953 Controlled reinfestation of treated soil; *Phytopathology* **43** 586–587

Flentje N T and Saksena H K 1957 Studies on *Pellicularia filamentosa* (Pat.) Rogers. II. Occurrence and distribution of pathogenic strains; *Trans. Br. Mycol. Soc.* **40** 95–108

Frederiksen T, Jorgensen C A and Neilsen O 1938 Undersgelser over kartoflens Rodfiltsvamp og dens Bekaempelse; *Tidsskr Planteavl* **43** 1–64

Frissel M J and Poelstra P 1979 The fate of mercury containing fungicides. In: *Soil disinfestation* (ed.) D Mulder (Amsterdam: Elsevier), 337–347

Gapter J K 1968 Mode of action of PCNB; *Diss. Abstr.* **B30** 931–932

Gibson I A S, Ledger M and Boehm E 1961 An anomalous effect of pentachloronitrobenzene on the incidence of damping-off caused by a *Pythium* sp; *Phytopathology* **51** 531–533

Gilman J C and Melhus I E 1923 Further studies on potato seed treatment; *Phytopathology* **13** 341–358

Grover R K and Chopra B L 1972 Factors affecting the toxicity of carboxin and oxycarboxin against *Rhizoctonia solani*; *Acta Phytopathol. Acad. Sci. Hung.* **7** 131–138

Grover R K and Chopra B L 1977 Seed, soil and foliage treatments with carboxin and oxycarboxin for control of *Rhizoctonia* species and their toxicity to other fungi; *Pesticides* **11** 38–41

Grover R K and Thareja R K 1983 Some bio-ecological factors affecting the toxicity of carbendazim to *Sphaceloma ampelina* causing grape vine anthracnose; *Trop. Plant Sci. Res.* **1** 25–30

Hammerschlag R S and Sisler H D 1972 Differential action of benomyl and methyl-2-benzimidazole carbamate (MBC) in *Saccharomyces pastorianum*; *Pestic. Biochem. Physiol.* **2** 123–131

Hans J K, Tyagi P D, Kataria H R and Grover R K 1981 The influence of soil and other physical factors on the antifungal activity of carbendazim against *Rhizoctonia solani*; *Pestic. Sci.* **12** 425–432

Horsfall J G 1956 *Principles of fungicidal action* (Waltham, Mass: Chronica Botanica Co.)

Hynes H J 1937 Studies on *Rhizoctonia* root-rot of wheat and oats; *Dep. Agric. N.S. Wales Sci. Bull.* **58** 42 pp.

Jackson L W R 1940 Effects of H-ion and Al-ion concentration on damping-off of conifers and certain causative fungi; *Phytopathology* **30** 563–579

Janssen J J 1929 Invloed der Bemesting op de Gezonheid van de Aardappel; *Tijdschr. Plantenziek* **35** 119–151

Jhooty J S and Bains S S 1972 Evaluation of different systemic and non-systemic fungitoxicants for the control of damping-off of mung (*Phaseolus aureus*) caused by *Rhizoctonia solani*; *Indian Phytopathol.* **25** 509–512

Jhooty J S and Bains S S 1973 Studies on *Rhizoctonia solani*. III. Reaction of various isolates to different fungitoxicants; *Indian J. Microbiol.* **13** 27–32

Jhooty J S and Grover R K 1971 *Rhizoctonia* root rot of cucurbits and its control in India; *Indian Phytopathol.* **24** 571–574

Kataria H R 1973 *Investigations on the chemical control of Rhizoctonia solani Kuhn*. Ph.D. Diss. Haryana Agricultural University, Hissar, India

Kataria H R and Dodan D S 1981 Pathogenesis of *Pythium butleri* on cowpea as influenced by two herbicides; *Z. PflKrankh. PflSchutz.* **88** 734–743

Kataria H R and Dodan D S 1982 The influence of two herbicides on the antifungal activity of some fungicides against *Pythium butleri* and *Rhizoctonia solani* causing damping-off of cowpea; *Pestic. Sci.* **13** 583–588

Kataria H R and Dodan D S 1983 Impact of two soil-applied herbicides on damping-off of cowpea caused by *Rhizoctonia solani*; *Plant Soil* **73** 275–283

Kataria H R and Grover R K 1975a Inhibition of infection structures of *Rhizoctonia solani* by fungitoxicants; *Z. Pflkrankh. PflSchutz.* **82** 226–232

Kataria H R and Grover R K 1975b Effect of chloroneb (1,4-dichloro-2,5-dimethoxy benzene) and pentachloronitro benzene on metabolic activities of *Rhizoctonia solani* Kuhn; *Indian J. Exp. Biol.* **13** 281–285

Kataria H R and Grover R K 1976a Effect of benomyl and thiophanate-methyl on metabolic activities of *Rhizoctonia solani* Kuhn; *Ann. Microbiol. (Inst. Pasteur)* **A127** 297–306

Kataria H R and Grover R K 1976b Fungitoxicity of mineral oils against *Rhizoctonia solani* causing damping-off of mung bean (*Phaseolus aureus*); *Ann. Appl. Biol.* **83** 79–85

Kataria H R and Grover R K 1976c Some factors affecting the control of *Rhizoctonia solani* by systemic and non-systemic fungicides; *Ann. Appl. Biol.* **82** 267–278

Kataria H R and Grover R K 1978a Comparison of fungicides for the control of *Rhizoctonia solani* causing damping-off of mung bean (*Phaseolus aureus*); *Ann. Appl. Biol.* **88** 257–263

Kataria H R and Grover R K 1978b Reversal of toxicity of some systemic and non-systemic fungitoxicants by chemicals; *Z. PflKrankh. PflSchutz.* **85** 76–83

Kataria H R and Sunder S 1983 Micronutrients modify activity of fungicides against *Rhizoctonia solani*; *Proc. 10th Int. Cong. Plant Prot. (Brighton)* **2** 587

Kataria H R, Yadav J P S and Grover R K 1981 Interactions of chemical fertilizers with seed dressing fungicides in controlling *Rhizoctonia solani*; *Z. PflKrankh. PflSchutz.* **88** 641–650

Kataria H R, Yadav O P, Tyagi P D and Grover R K 1976 Fungitoxic action of pyracarbolid against *Rhizoctonia solani*; *Z. PflKrankh. PflSchutz.* **83** 416–421

Kernkamp M F, DeZeeuw D J, Chen S M, Ortega B C, Tsiang C T and Khan A M 1952 Investigations on physiologic specialization and parasitism of *Rhizoctonia solani*; *Minnesota Agric Exp. Sta. Tech. Bull.* **200** 36 pp.

Khan S U 1980 *Pesticides in the soil environment* (Amsterdam: Elsevier)

Kluge E 1969 Der einfluss der bodenreaktion auf den abbau und die wirkungsdauer von thiuram, ferbam and captan im Boden. *Arch. PflSchutz.* **5** 263–271

Kühn J G 1858 Die Krankheiten der Kulturegewachse, ihre ursachen und ihre Verhutung. Gustav Bosselmann, Berlin.

Last F T 1952 The use of tetra- and penta- chloronitrobenzenes in the control of *Botrytis* disease and *Rhizoctonia* attack on lettuce; *Ann. Appl. Biol.* **39** 557–568

Leach L D and Garber R H 1970 Control of *Rhizoctonia*; In *Rhizoctonia solani*, biology and pathology (ed.) J R Parameter Jr (Berkeley, Los Angeles and London: University of California Press) pp 189–199

Leach L D, Garber R H and Lange W H 1959 Cotton seed treatment trials in California, 1954–1958, with special reference to specific fungicides; *Plant Dis. Rep. Suppl.* **259** 213–221

Leach L D, Garber R H and Tomsoff W J 1960 Selective protection afforded by certain seed and soil fungicides; *Phytopathology* **50** 643–644

Lewis J A and Papivizas G C 1977 Factors affecting *Rhizoctonia solani* infection of soybeans in the greenhouse; *Plant Dis. Rep.* **61** 196–200

Livingston C H, Oshima N and Marrill C 1962 Evaluation of terraclor as a control measure for *Rhizoctonia* disease of potatoes; *Phytopathology* **52** 18

Lohman J 1925 Stimulatory effects of chemical compounds on germination of the potato tubers; *Landw. Jahrb.* **1** 1–44

Lukens R J 1971 *Chemistry of fungicidal action* (Berlin: Springer Verlag)

Luttrell E S 1962 Rhizoctonia blight of tall fescue grass *Plant Dis. Rep.* **46** 661–664

Lyr H and Werner P 1982 On the mechanism of action of the fungicide chloroneb; *Pestic. Biochem. Physiol.* **18** 69–76

Maier C R 1962 Response of selected *Rhizoctonia solani* isolates to different chemicals in cultural tests; *Phytopathology* **52** 19

Malhan I, Tyagi P D and Grover R K 1975 Physical factors affecting toxicity of benomyl to *Rhizoctonia solani* in vitro; *Indian Phytopathol.* **28** 491–494

Manji B T, Bose E, Ogawa J M and Uriu K 1971 Effect of copper on the activity of benomyl; *Phytopathology* **61** 1323

Martinson C A 1963 Inoculum potential relationship of *Rhizoctonia solani* measured with soil microbiological sampling tubes; *Phytopathology* **53** 634–638

Menon K P V, Nair U K and Pandalai K M 1952 Influence of waterlogged soil conditions on some fungi parasitic on the roots of the coconut palm; *Indian Coconut J.* **5** 71–79

Mills H A, Barker A V and Maynard D N 1974 Ammonia volatilization from soils; *Agron J.* **66** 355–358

Munnecke D E 1972 Factors affecting the efficacy of fungicides in soil; *Ann. Rev. Phytopathol.* **10** 375–398

Neergaard P 1958 Infection of Danish seeds by *Rhizoctonia solani* Kuehn; *Plant Dis. Rep.* **42** 1276–1278

Papavizas G C 1970 Colonization and growth of *Rhizoctonia solani* in soil. In *Rhizoctonia solani, biology and pathology* (ed.) J R Parmeter Jr (Berkeley, Los Angeles and London: University of California Press) 108–124

Papavizas G C and Davey C B 1961 Saprophytic behaviour of *Rhizoctonia* in soil; *Phytopathology* **51** 693–699

Parmeter J R Jr (ed.) 1970 *Rhizoctonia solani, biology and pathology* (Berkeley, Los Angeles and London: University of California Press)

Parmeter J R Jr and Whitney H S 1970 Taxonomy and nomenclature of the imperfect state. In *Rhizoctonia solani, biology and pathology* (ed.) J R Parmeter Jr (Berkeley, Los Angeles and London: University of California Press) 7–19

Pesek J T Jr 1964 Comparing efficiency of nitrogen fertilizers. In *Fertilizer nitrogen—its chemistry and technology* (ed.) V Sauchelli (New York: Reinhold Publ. Co.) 365–391

Pinckard J A and Standifer L C 1966 An apparent interaction between cotton herbicidal injury and seedling blight; *Plant Dis. Rep.* **50** 172–174

Quanjer H M 1916 Over de betekenis van het poortoed voor de verspreiding van aardappel ziekten en over de voordeclen eener behandling met sublimat; *Med. Pijks. Bosch.* **9** 94–122

Raeder J M and Hungerford C W 1922 The effects of pre-sprinkling with water upon the efficacy of certain potato seed treatments for the control of *Rhizoctonia*; *Phytopathology* **12** 447

Ragsdale N N and Sisler H D 1970 Metabolic effects related to fungitoxicity of carboxin; *Phytopathology* **60** 1422–1427

Richards B L 1921 A dry rot canker of sugarbeets; *J. Agric Res.* **22** 47–52

Richards B L 1923 Soil temperature as a factor affecting the pathogenicity of *Corticium vagum* on the pea and the bean; *J. Agric. Res.* **25** 431–451

Richter H and Schneider R 1953 Untersuchungen zur morphologischen und biologischen Differenzierung von *Rhizoctonia solani* K; *Phytopathol. Z.* **20** 167–226

Rolfs F M 1904 Potato failures. A second report. *Colorado Agric. Exp. Sta. Bull.* **91** 33 pp.

Rose R C 1925 Treating seed potatoes with hot formaldehyde; *Minn. Agric. Exp. Div. Spec. Circ.* **22** 3 pp.

Rosen H R 1924 The control of damping-off of cotton seedlings by the use of Uspulum; *Science* **60** 384

Roth L F and Riker A J 1943 Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by *Pythium* and *Rhizoctonia*; *J. Agric. Res.* **67** 273–293

Rushdi M and Jeffers W F 1956 Effect of some soil factors on efficiency of fungicides in controlling *Rhizoctonia solani*; *Phytopathology* **46** 88–90

Saksena H K and Vaartaja O 1960 Description of new species of *Rhizoctonia*; *Can. J. Bot.* **38** 931–943

Saksena H K and Vaartaja O 1961 Taxonomy, morphology, and pathogenicity of *Rhizoctonia* species from forest nurseries; *Can. J. Bot.* **39** 627–647

Sauchelli V 1964 The role of nitrogen in agriculture. In *Fertilizer nitrogen—its chemistry and technology* (ed.) V Sauchelli (New York: Reinhold Publ. Co.) 1–9

Schaal L A 1935 Rhizoctoniosis of potatoes grown under irrigation; *Phytopathology* **25** 748–762

Schmiederknecht M 1960 Feuchtigkeit als standortfaktor fur mikroskopische Pilze; *Z. Pilzk.* **25** 69–77

Schneider R 1953 Untersuchungen uber Feuchtigkeit-sanspruche parastischer Pilze; *Phytopathol. Z.* **21** 63–78

Schnitzer M and Khan S U 1972 *Humic substances in the environment* (New York: Marcell Dekkar)

Shatla M N and Sinclair J B 1962 Tolerance to pentachloronitrobenzene and pathogenicity correlated in naturally occurring isolates of *Rhizoctonia solani*; *Phytopathology* **52** 752

Sinclair J B 1960 Reaction of *Rhizoctonia solani* isolates to certain chemicals; *Plant Dis. Rep.* **44** 474–477

Singh R S and Singh B 1957 Incidence of seedling-rot, root-rot, and wilt of *Cyamopsis psoralioides* under manured and unmanured conditions; *Agra Univ. (India) J. Res. (Sci.)* **6** 7–14

Small T 1927 Rhizoctonia "foot rot" of the tomato; *Ann. Appl. Biol.* **14** 290–295

Sommartaya 1980 *Role of host on the efficacy of fungicides in plant disease control.* Ph.D. Diss. Haryana Agricultural University, Hissar, India

Stevenson F J 1964 Soil nitrogen. In *Fertilizer nitrogen—its chemistry and technology* (ed.) V Sauchelli (New York: Reinhold Publ. Co.) 18–39

Sunder S 1982 *Influence of organic manures and clay minerals on the efficacy of fungicides against Rhizoctonia solani*; M.Sc. Thesis, Haryana Agricultural University, Hissar, India.

Talbot P H B 1970 Taxonomy and nomenclature of the perfect state. In *Rhizoctonia solani, biology and pathology* (ed.) J R Parmeter Jr (Berkeley, Los Angeles and London: University of California Press) 20–31

Taneja M and Grover R K 1982 Efficacy of benzimidazole and related fungicides against *Rhizoctonia solani* and *R. bataticola*; *Ann. Appl. Biol.* **100** 425–433

Teuscher H and Adler R 1960 *The soil and its fertility* (New York: Reinhold Publ. Co.)

Thompson L M and Troeh F R 1979 *Soil and soil fertility* (New Delhi: Tata McGraw-Hill Book Co.)

Thaxter R 1890 The smut of onions (*Urocystis cepulae* Frost.); *Ann. Rep. Connecticut Agric. Exp. Sta.* **1889** 129–153

Tisdale S L and Nelson W L 1960 *Soil fertility and fertilizers* (New York: Macmillan)

Vasudeva R S and Ashraf M 1939 Studies on the root-rot disease of cotton in the Punjab. VII. Further investigation of factors influencing incidence of the disease; *Indian J. Agric. Sci.* **9** 595–608

Venkataramaiah G H 1964 Evaluation of fungicides in the control of post-emergence damping-off of coffee seedlings caused by *Rhizoctonia solani*; *Indian Coffee* **28** 257–262

Weinhold A R 1977 Population of *Rhizoctonia solani* in agricultural soils determined by a screening procedure; *Phytopathology* **67** 566–569

Wenham H T, Mackintosh B L and Bolkan H A 1976 Evaluation of fungicides for control of potato black scurf; *N. Z. J. Exp. Agric.* **4** 97–101

Winston J R 1913 Effect of the steam-formalin treatment on certain soil organisms; *Phytopathology* **3** 74

Worthing C R 1979 *The pesticide manual* (London: British Crop Prot. Council)

Wyse D L, Meggitt W F and Penner D 1976 Effect of herbicides on the development of root rot on navy bean; *Weed Sci.* **24** 11–15

Zaleski K and Blaszczak W 1954 Wstepne doswidozenia nad patologia Szkodliwoscia i niektorymi sposobami zwalczania rizoktoniozy Ziemniakow (*Rhizoctonia solani* Kuehn); *Roczn. Nauk Roln.* **69** 529–556

Zentmyer G A 1944 Inhibition of metal catalysis as a fungistatic mechanism; *Science* **100** 294–295

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 433–436.
© Printed in India.

Cylindrocarpon species associated with mycotic keratitis

C BOOTH, Y M CLAYTON* and M USHERWOOD**

Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey TW9 3AF, UK

* Institute of Dermatology, Lisle Street, Leicester Square, London

** Department of Botany, University College, London, UK

Abstract. Records of mycotic keratitis caused by *Cylindrocarpon* species are increasing. *C. vaginae* is described as new and related to *C. lichenicola*.

Keywords. Mycotic keratitis; vaginae; *C. lichenicola*, *Fusarium solani*.

In recent years a large number of cases of oculomycosis have been reported, mainly caused by *Fusarium solani* (Mart.) Sacc. (Jones *et al* 1969)

In addition cases have also been reported of mycotic keratitis caused by *Cylindrocarpon lichenicola* = *C. tonkinensis* (Hawksworth 1979) or by *Cylindrocarpon* species. These isolates have a superficial appearance similar to that of some strains of *F. solani* in culture but they are easily distinguished on the morphology of both the conidia and the chlamydospores. Cases of *C. lichenicola* have been reported by Laverde *et al* (1973) from Venezuela and by Matsumoto *et al* (1979) from Japan. Both these infections followed contamination of the eye by muddy water. These isolates have been checked and found to be typical of *C. lichenicola*.

Compared to infections of the eye caused by *F. solani* those caused by the *Cylindrocarpon* species are rare. Liesegang and Forster (1980) reported the results of scraping 137 corneal ulcers caused by fungi. Of these 76 were caused by *F. solani* and only one by a *Cylindrocarpon* species. The latter was not named.

In January 1984 we received a culture from the Institute of Opthalmology, London, which had been isolated from the eye of a British farmer following an injury. This isolate resembled superficially the young isolates of *C. lichenicola* but the spores never reached the same dimensions or the multiple septation of this species, also the formation of the chlamydospores was different. Most isolates of *C. lichenicola* produce abundant chlamydospores which are for the most part formed terminally on short lateral branches and are usually 2–4 celled. The chlamydospores in this isolate although occasionally formed in a similar manner, are on average smaller and more globose and some have a thin-walled basal cell which produces a small clump of chlamydospores at the apex—a feature not found in *C. lichenicola*.

In a search through the medical mycology literature we became struck by the similarity of these chlamydospores to those figured by Nannizzi (1926) which he called *Diplosporium vaginae*. The conidia he figured appeared to be more pointed at each end than in our isolate but it is well known that in those species with thin-walled hyaline spores the effect of the mounting media can produce this appearance, especially if lactic acid is used. We therefore made an effort to obtain material of Nannizzi's species from the herbarium at the University of Siena, Italy, but Dr V De Dominicis, the Curator at Siena, informed us that the material was not now available. This is often the case of many of the species that were described from cultures in the early days of fungal

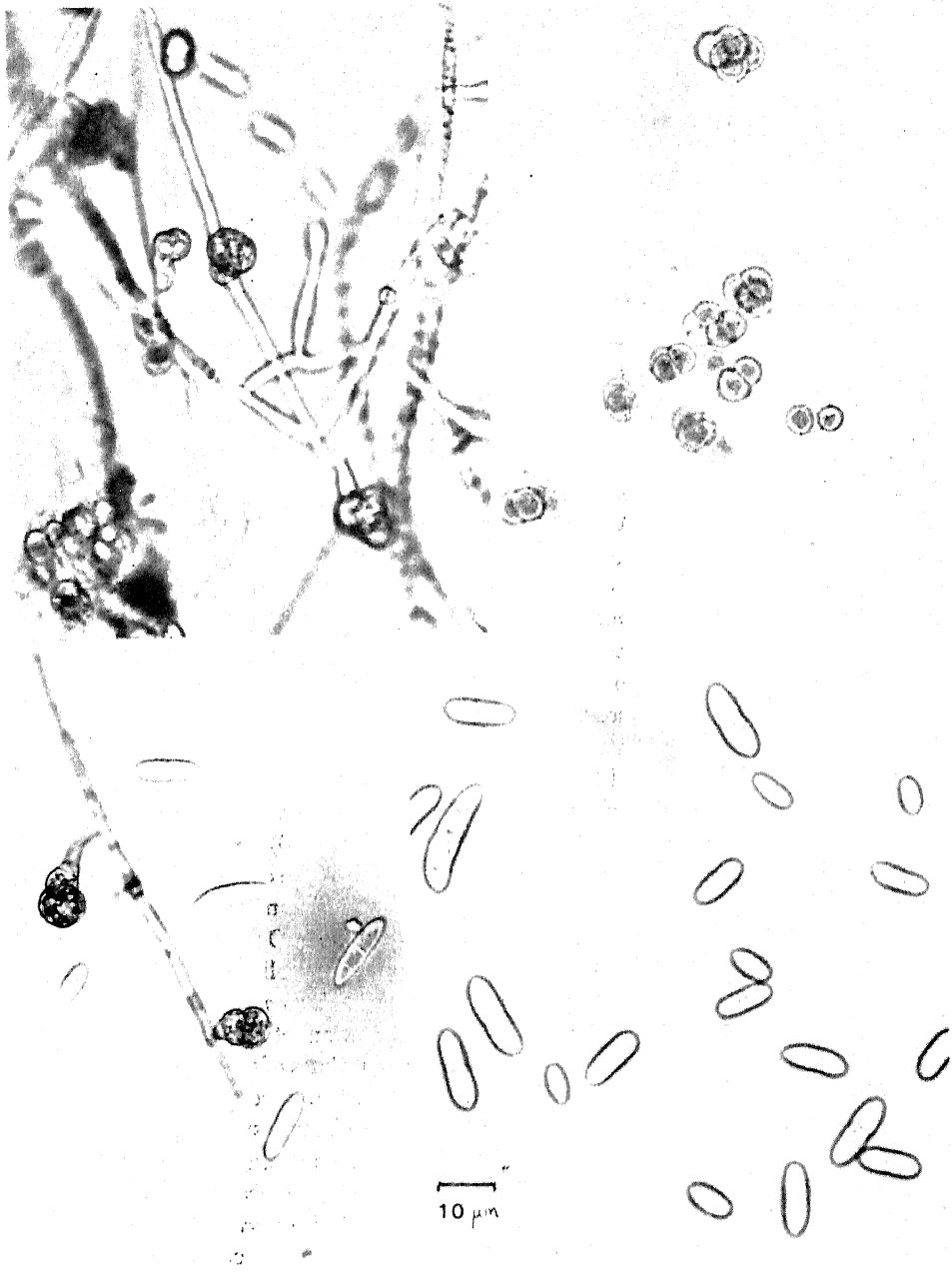


Figure 1. *Cylindrocarpon vaginae*—conidia, conidiophore and chlamydospores.

cultures and before culture collections became standard reference centres.

In any case the genus *Diplosporium* would be no longer tenable for this species. Hughes (1958) stated that the type of *Diplosporium* Link (1824) was the same as *Oedemium* Link (1924) described earlier and that in *Diplosporium nigrescens* Link the type was an obligate synonym of *Oedemium didymum* (Schm.) Hughes. It was therefore

decided to refer our isolate to the genus *Cylindrocarpon* and although we cannot without doubt confirm that it is identical to Nannizzi's species we are retaining the epithet '*vaginae*' in recognition of his early work.

Ten single spore isolates on potato dextrose agar had a wide range of temperature for growth *i.e.* between 18 and 32°C but no growth occurred at 36°C or over. Growth at 30°C produced abundant creamy white floccose aerial mycelium which covered the agar surface of a 9 cm plate after 7 days. The aerial mycelium at the surface of the agar then gradually becomes pale yellow or greyish-brown. From below the colourless agar becomes reddish to dark brick or brown vinaceous (Rayner 1970) from the 7th day onwards beginning at the centre and extending outwards.

Conidiophore formation begins towards the edge of the colony after 3–4 days. They arise as lateral branches which may form only a foot-cell with an apical phialide or they may form well developed but sparsely branched conidiophores, 20–80 μm long, with each branch terminating in a single phialide. These are subulate 18–32 μm long by 4–4.5 μm at the base. Conidia are fusiform to oval, 0–1 septate rather more rounded

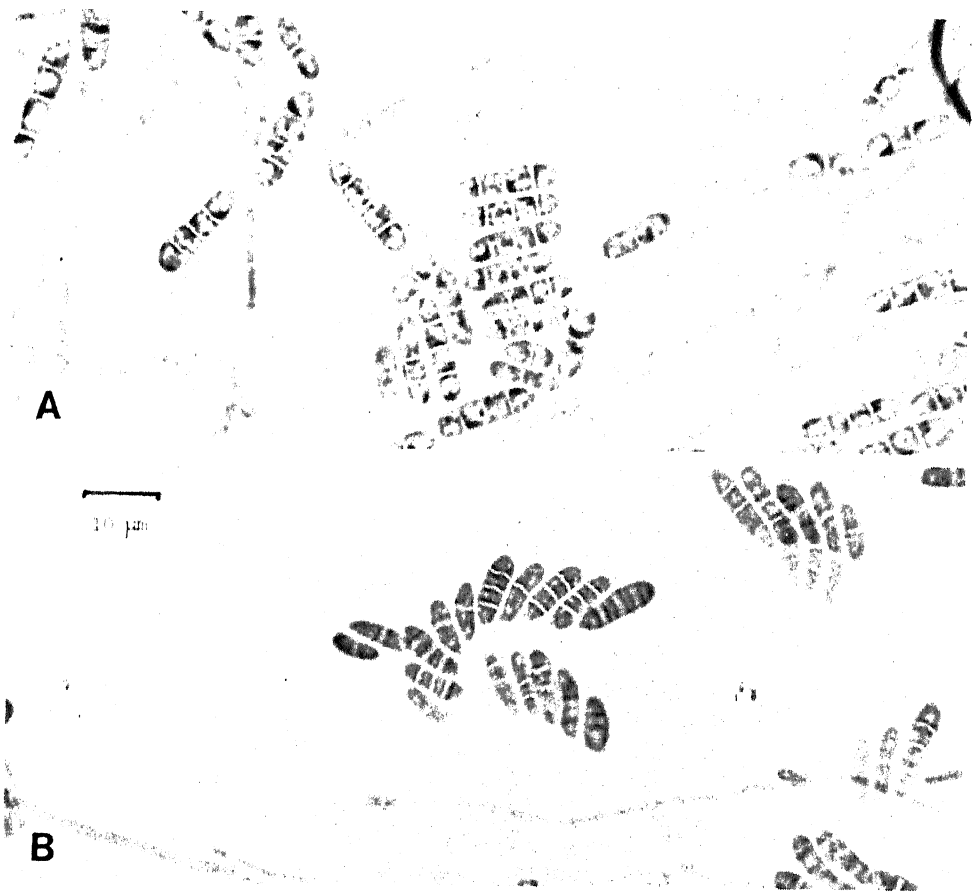


Figure 2. **A.** Conidia ex original culture of *Cylindrocarpon tonkinensis* Bugn. (IMI 55920). **B.** Isolate of *C. lichenicola* (*C. tonkinensis*) from ulcerated human tissue. UK (IMI 74423).

at the apex than the base. Occasional spores become 2 or even 3-septate and measure 20–24 × 4–5 μm.

Chlamydospores are the only other asexual spore type formed and these are abundant. Occasionally they form intercalary or terminally, singly or in short chains, but for the most part they are formed on a globose vesicle which terminates a short lateral branch. These lateral branches are 8–36 μm long terminating in a globose vesicle 6–7 μm in diameter from which is formed 1–6 chlamydospores. As the number increases they arrange themselves like a cluster of droopes as found in a blackberry—a method of formation not previously found in this genus of fungi. The thick-walled chlamydospores are globose or variously flattened if occurring in a cluster. They are smooth walled or occasionally covered with short spines and they measure 8–10 (8–12) μm in diameter.

Cylindrocarpon vaginae sp. nov. Diagnosis *In culture: Mycelium* album dein pallidus eburneus, caespitosus. Hyphis 3–6 μm crassis. *Conidiophora* simple vel ramosus 20–80 μm longus phialidibus subulatis terminantia, 8–32 × 4–5 μm. *Conidia* hyalina, fusiformia brevites pedunculata 1–2 celluloe raro 3–4 cellularae, 20–24 × 4–5 μm. *Chlamydosporae* globosae, hyalinae 9–12 μm in diam. pariete crassa, laevi vel spiniformi terminaliter formatae, simplices vel polymorphes haud raro pedunculatae, saepe vesicula globulosa 2- vel pluricellulari. Type IMI 281978. Living cultures in Culture Collection, CMI and CBS, Baarn.

References

Jones B R, Jones D B, Lim A S M, Bron A J, Morgan G and Clayton Y M 1969 Corneal and intra-ocular infection due to *Fusarium solani; Trans. Ophtholmol. Soc. U. K.* **89** 757–779

Hawksworth D L 1979 The lichenicolous Hyphomycetes; *Bull. Br. Mus. Nat. Hist., Bot.* **6** 273

Hughes S J 1958 Revisiones Hyphomycetum Aliquot cum Appendice de Nominibus Rejiciendis; *Can. J. Bot.* **36** 714, 727–836

Laverde S, Moncada L H, Restrepo A and Vera C L 1973 Mycotic keratitis, 5 cases caused by unusual fungi; *Sabouraudia* **11** 119–123

Liesegang T J and Forster P K 1980 Spectrum of microbial keratitis in south Florida; *Am. J. Ophthalmol.* **90** 38–47

Matsumoto T, Masaki J and Okabe T 1979 *Cylindrocarpon tonkinense:* as a cause of keratomycosis: *Trans. Br. Mycol. Soc.* **72** 503–504

Nannizzi A 1926 Osservazioni sui dimorfismo degli elementi riproduttori agamici in un nuovo *Diplosporium* (*D. vaginae* sp. n.) ed in altri generi di Hyphales; *Atti. R. Accad. Fisiocrit.* Siena IX, **17** 491–499

Rayner R W 1970 *A mycological colour chart* Commonwealth Mycological Institute London and British Mycological Society

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 437–451.
© Printed in India.

Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice

M MIYAJI and K NISHIMURA

Department of Pathogenic Fungi, Research Institute for Chemobiodynamics, Chiba University, Inohana, Chiba 280, Japan

Abstract. The conidial ontogenesis and the pathogenicity of the pathogenic black yeasts for mice are described. Scanning electron microscopy of conidiogenesis, physiological and biochemical characteristics and histological findings of infected mice are described in this paper.

Keywords. Conidial ontogenesis; black yeast; neurotropism; GC content.

1. Introduction

Most microbiologists might not be familiar with pathogenic black yeasts which are different from pathogenic white yeasts such as *Candida* spp. and *Cryptococcus neoformans*. Depending on the culture conditions, their mycelial colony converts to a yeast, thus they are dimorphic. There are five species of black yeasts, classified in two genera, *Exophiala* and *Hortaea*. They are *Exophiala dermatitidis*, *E. jeanselmei*, *E. moniliae*, *E. spinifera* and *Hortaea werneckii*. These fungi are well authenticated as the causative agents of chromomycosis, phaeohyphomycosis, mycetoma, mycotic keratitis and tinea nigra.

The pathogenic black yeasts are an important fungal group in the field of medical mycology. Even though most of the black yeasts have been isolated from non-life threating cutaneous and subcutaneous lesions, *E. dermatitidis* is neurotropic and has caused death in approximately one third of the patients infected with this fungus.

As of yet, no teleomorph has been found in the black yeasts. Until now, many studies on the conidial ontogenesis of the pathogenic black yeasts have been made by mycologists (Carrión 1950; Carrión and Silva 1955; Borelli 1955; Cooke 1962; Emmons 1966; Schol-Schwarz 1968; Conant *et al* 1971; Oujezdsky *et al* 1973; de Hoog 1977; Emmons *et al* 1977; McGinnis 1977, 1978; McGinnis and Padhye 1977; Cole 1978; Padhye 1978). However, the taxonomy of the black yeasts has remained controversial. Recently, observations of the conidial ontogenesis of the black yeasts through electron microscopy or time-lapse photomicrography have brought on one new discovery after another and, as a result, the study of their taxonomy and phylogeny is coming to a turning point.

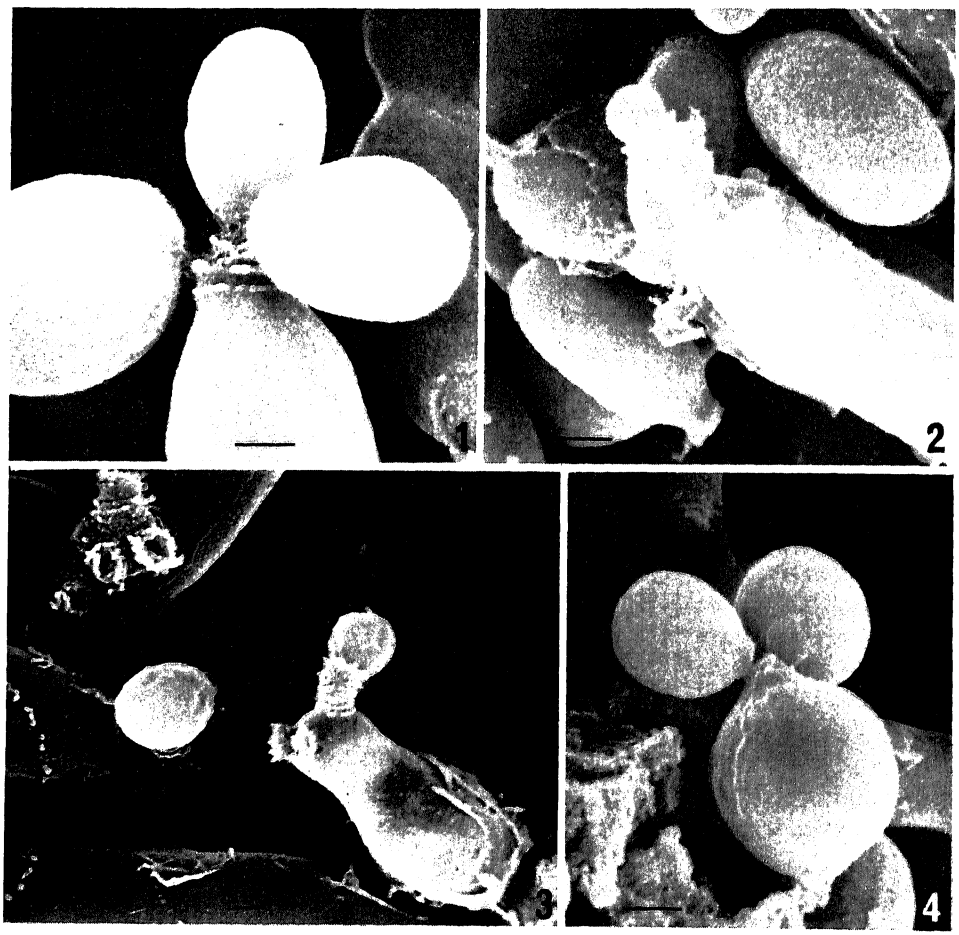
The authors have been investigating the conidial ontogenesis of the pathogenic black yeasts by scanning electron microscopy (Nishimura and Miyaji 1982, 1983, 1984) and their pathogenicity for mice (Nishimura and Miyaji 1983).

In this paper our previous investigation on the conidial ontogenesis and the pathogenicity of the pathogenic black yeasts for mice are described. Additionally we present new information on the susceptibility of mice infected with *E. jeanselmei* and *E. spinifera*.

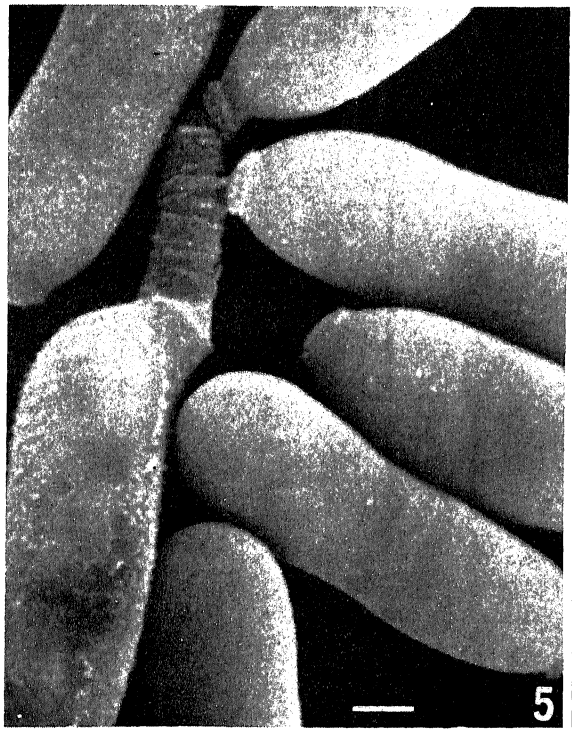
2. Conidial ontogenesis

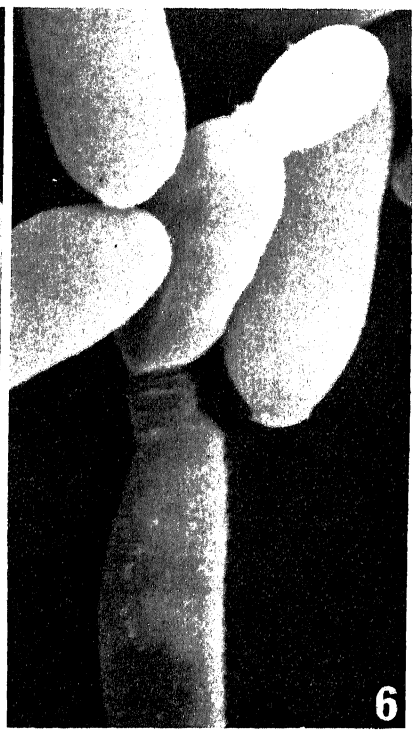
2.1 *Exophiala* Carmichael

2.1a *Exophiala dermatitidis* (Kano) de Hoog (figures 1–4): One to five tips (1·1 μm in diameter, 0·6 μm in length on the average) are found at the apices of bottle- or jar-shaped conidiogenous cells, and annellations looking like frills are observed on the tips. Annellated tips of *E. dermatitidis* are shorter than those of the other *Exophiala* species. Their average size is 0·6 to 0·9 μm in length and 1·0 to 1·2 μm in diameter. Annellations (1–9) are formed on the tip. The annellations are parallel and sometimes look like multiple collarettes. Annelloconidia are also produced from small projections with annellations on the hyphal wall. These findings are very similar to those of *Exophiala salmonis* which is the type species of the genus *Exophiala*. In *E. salmonis* (figures 5, 6) its



Figures 1–4. **1.** *E. dermatitidis* IFM 4828. Annellations are clearly observed. **2.** *E. dermatitidis* IFM 4830. Flaring tip with annellations. **3.** *E. dermatitidis* IFM 4826. Three tips with annellations are formed on an annellide. **4.** *E. dermatitidis* IFM 4826. Mother cell producing annelloconidia. (Bar is 1 μm).





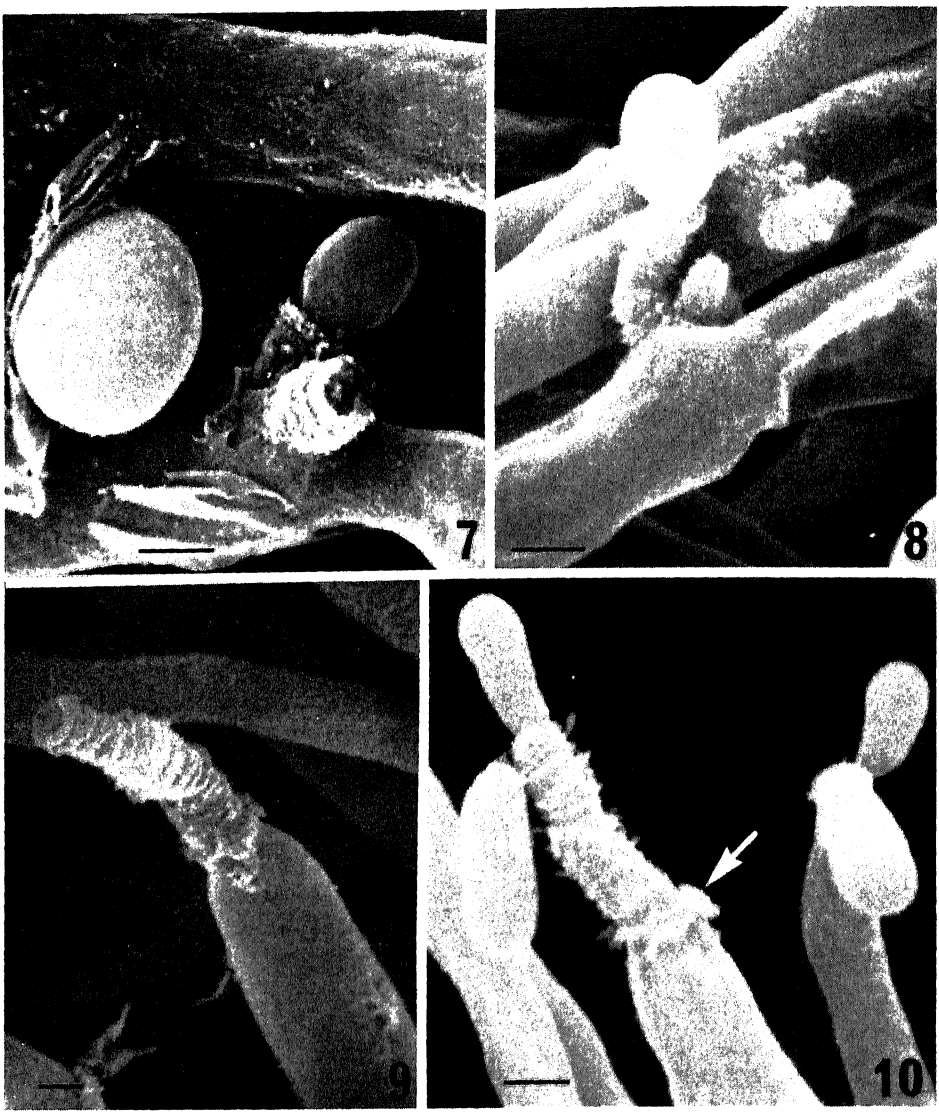
Figures 5–6. **5.** *E. Salmonis* IFM 4884. Annellide with a comparatively long tip. **6.** *E. salmonis* IFM 4884. Annellations between a primary and secondary annellide. (Bar is 1 μm).

hyphae also function as annellides and produce annelloconidia. Occasionally, a secondary or tertiary annellide is formed from a primary or secondary annellide. Such a phenomenon is also observed in *E. dermatitidis*, *E. jeanselmei*, *E. moniliae* and *E. spinifera*. Annellations are also observed at the connecting part between two annellides. Annelloconidia are spherical, oval or rugby ball-shaped. Conidia also produce conidia secondarily. In its yeast form one to five projections with a few annellations appear on the mother conidia. The conidiogenesis of *E. dermatitidis* is only annellidic (Nishimura and Miyaji 1982, 1983).

2.1b *Exophiala jeanselmei* (Langeron) McGinnis et Padhye (figure 7): There are no major differences in scanning electron microscopical findings between *E. jeanselmei* and *E. dermatitidis*. Its conidiogenesis is only annellidic.

2.1c *Exophiala moniliae* de Hoog (figure 8): *Exophiala moniliae* was first isolated as a new species from a branch of the *Quercus* sp. by de Hoog (1977). It was isolated from a patient with phaeohyphomycosis by McGinnis *et al* (1981).

Conidiogenous loci (1–5) arise from an annellide and form annellated tips. They are sometimes in a cluster. The annellated tips are more slender than those of the other *Exophiala* species. The size of the tips is 0·7 × 1·2 μm on the average. As these annellated tips grow, they show a tendency to curve towards the direction of the mycelial growth.



Figures 7–10. **7.** *E. jeanselmei* IFM 4853. Two annellated tips on a hypha. An annelloconidium occurs from one of them. **8.** *E. moniliae* IFM 4881. Slender annellated tips showing a tendency to curve towards the direction of mycelial growth. **9.** *E. spinifera* IFM 4883. Long annellated tip with many annellations. **10.** *E. spinifera* IFM 4883. First annellation (arrow) is conspicuous compared to successive annellations. (Bar is 1 μm).

The width between the annellations with short or rudimentary frills are very close. The average number of annellations is eight.

2.1d *Exophiala spinifera* (Nielsen et Conant) McGinnis (figures 9, 10): *Exophiala spinifera* was first isolated from the nasal granuloma of a patient by Nielsen and Conant (1968). They termed it *Phialophora spinifera.* McGinnis (1977) transferred it to the genus *Exophiala.*

Annellated tips arise singly from conidiogenous cells. They are the longest of all the species of *Exophiala*. They often have more than thirty annellations. The annellations are sometimes formed irregularly. As in the other *Exophiala* species, the annelloconidia usually aggregate in a small ball at the annellated tips. However, in some cases, annelloconidia attach along the annellated tips. Such conidia look as if they were produced sympodially. Annellated tips sometimes change their growth direction during the development, and as a result they become zigzag-shaped. In this species the frill of the first annellation is often long. The size of the annellated tip is 0·9 × 2·3 μm on the average. Neither sympodulae nor phialides have been observed.

2.2 *Hortaea* Nishimura et Miyaji

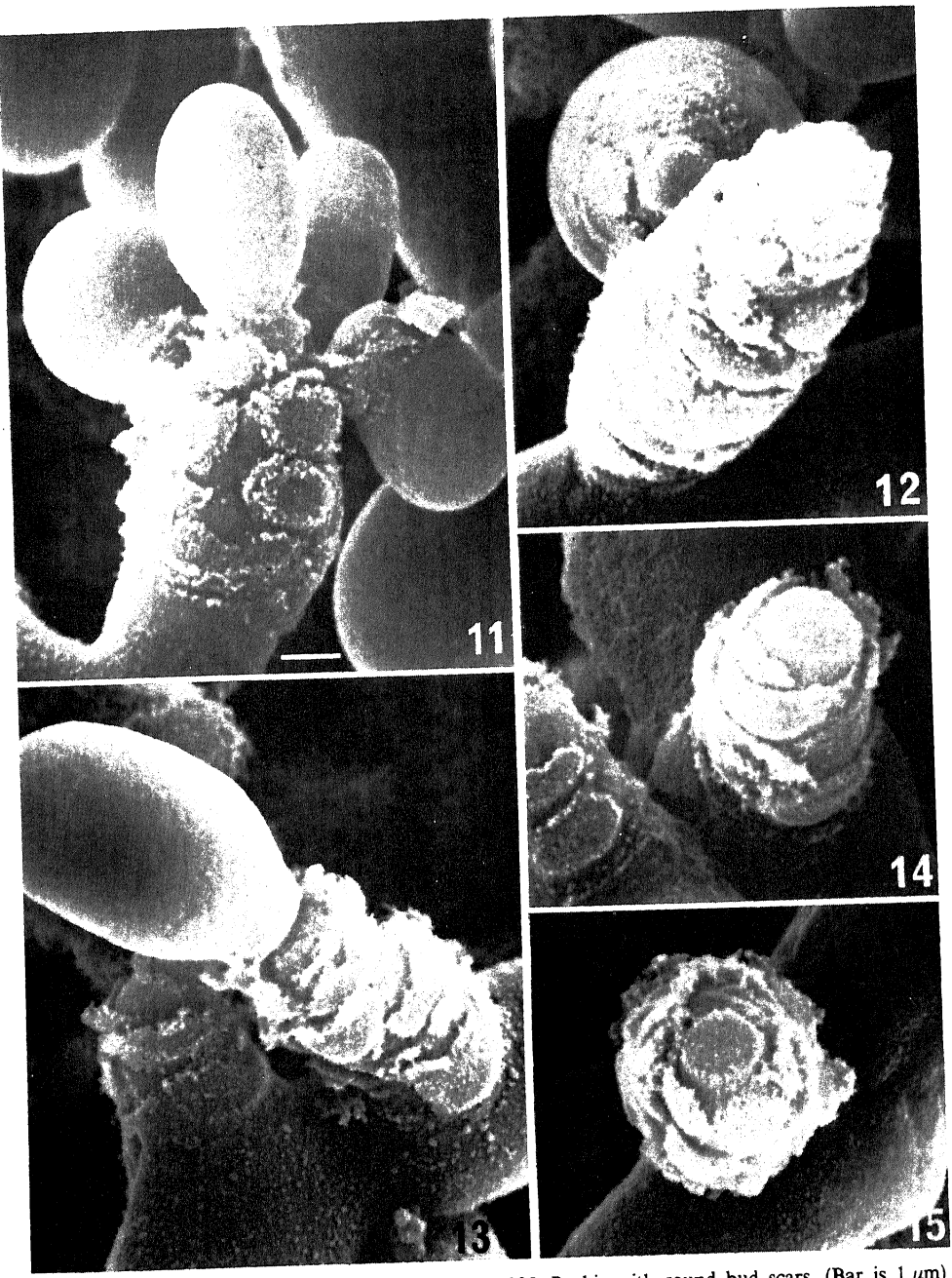
2.2a *Hortaea werneckii* (Horta) Nishimura et Miyaji (figures 11–15): The genus *Hortaea* was proposed by Nishimura et Miyaji (Nishimura and Miyaji 1984) to accommodate *Cladosporium werneckii* Horta (Horta 1921) in 1984. The type species is *Hortaea werneckii*.

The conidial ontogenesis of *H. werneckii* consists of a combination of sympodial and annellidic conidiogenesis. Its sympodial one is particularly unique. Conidiogenous cells elongate and swell in accordance with producing terminal conidia. In a few conidiogenous cells, round bud scars are sympodially arranged on the rachises (figure 11). Denticles are not detected on the surface. Most of the conidiogenous cells have remnants of bud scars shaped like sickles or half moons (figures 13, 14). Some sympodulae are covered with remnants of scaly bud scars (figure 12). Bud scars vary in shape from cell to cell. One or two round bud scars are observed at the apex of rachises (figures 14, 15). Conidia occur at higher levels in a clockwise direction (figures 14, 16A).

Imaginatively, a spiral can be drawn on a conidiogenous cell by tracing the centres of the scars upwards (figure 17). We designate the angle of revolution between a bud scar and the next formed one as a divergence. If the divergence of the fungus is a 2/5 cycle, bud scars are formed as shown in figures 15, 16B, C. When the pitch of the spiral is almost equal to the radius of the scars, the round bud scars might be arranged in lengthwise rows (figures 11, 17A). When the pitch of the spiral is smaller than the radius of the scars, a new conidium appears by breaking through the upper part of a previously formed bud scar and as a result, half moon-shaped remnants of scars are left on the conidiogenous cell (figures 16A, 18). When the pitch is much smaller and the divergence is a 3/8 cycle, scaly bud scars overlap each other and the conidiogenous cells look like a pinecone (figures 12, 17B). When the radius of the spiral is small, a new conidium emerges by breaking through a part of the next old scar and as a result lunate or falcate bud scars are arranged spirally (figures 13, 18). In other words they are in sympodial arrangement. There are a few conidiogenous cells with annellations in both the mycelial and yeast forms. If the radius of the spiral is almost equal to zero, annellations are formed and the annellidic anamorph of this species is considered to be a homology of the sympodial anamorph. According to Grove *et al* (1973) *Cladosporium* (*Hortaea*) *werneckii* is holoblastic, unlike *E. dermatitidis*, which is enteroblastic (Grove *et al* 1973).

3. Physiological characteristics and GC contents (mol % guanine plus cytosine)

Exophiala dermatitidis grows at 42°C. Only two out of 18 cultures of *E. jeanselmei* isolated from living circumstances can grow at 37°C but not at 40°C. Three out of 6



Figures 11–15. **11.** *H. werneckii* IFM 4885. Rachis with round bud scars. (Bar is 1 μm) **12.** *H. werneckii* IFM 4885. Rachis looking like a pine cone. **13.** *H. werneckii* IFM 4885. Alternating lunate scars on a rachis. **14.** *H. werneckii* IFM 4885. Bud scars looking like petals. **15.** *H. werneckii* IFM 4885. Rachis looking like a rose. Two bud scars are observed at an apex.

cultures of *E. moniliae* grow at 40°C but not at 42°C. *E. spinifera* grow at 37°C but its growth is suppressed at 40°C. *H. werneckii* grows slightly at 37°C but not at 40°C.

E. dermatitidis assimilates potassium nitrate slightly in the test using bact-yeast

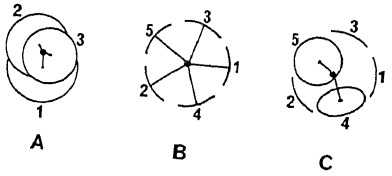


Figure 16. Bud scars are labelled 1–5 to indicate their order of formation.

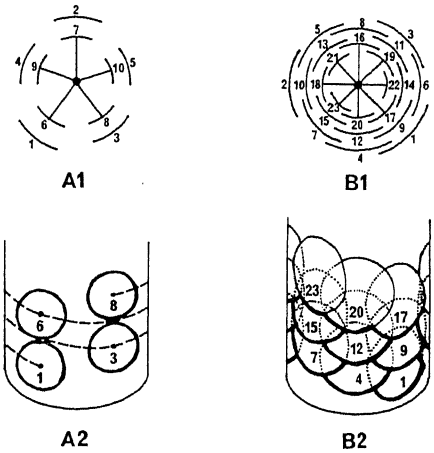


Figure 17. Bud scars are formed in the designated numerical order.

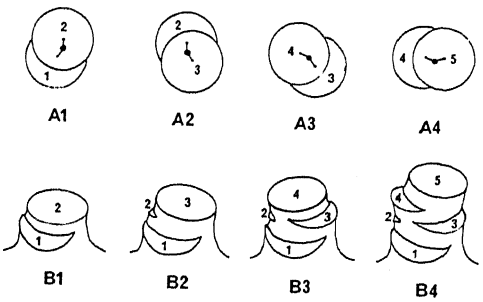


Figure 18. Bud scars are labelled 1–5 to indicate their order of formation.

nitrogen base. The other *Exophiala* species and *H. werneckii* assimilate potassium nitrate. *H. werneckii* hydrolyzes skim milk, but the *Exophiala* species do not. All the cultures used assimilate all carbon compounds tested except lactose and hydrolyze urea.

The GC contents of *E. dermatitidis*, *E. jeanselmei*, *E. moniliae* and *E. spinifera* are 58·6 59·5, 59·0 and 59·7 %, respectively. Those of *E. salmonis* and *E. pisciphila* are 52 and 51·5 %, respectively.

As mentioned above, the GC contents of the *Exophiala* species pathogenic for human

are considerably different from those of the *E. salmonis* and *E. pisciphila* which are pathogenic for fish. The GC content of *H. werneckii* is 54·0% (table 1).

4. Pathogenicity for mice

4.1 *Exophiala dermatitidis*

The virulence of *E. dermatitidis* to ddY male mice was examined using five cultures of the species (Nishimura and Miyaji 1982). Fifty male ddY mice were divided into five groups consisting of ten each. In the mice inoculated intravenously with 5×10^6 yeast cells of the culture isolated from a humidifier (IMF 4826), two died on the 6th and 7th day. There were no dead mice in the other four groups of mice for 30 days. The brains of the two dead mice were severely infected. A large number of polymorphonuclear leucocytes accumulated around short hyphae and yeast cells, forming many micro-abscesses. A small number of microgranulomas with yeast cells were also observed in the brain tissues. Symptoms of central nervous system involvement (torticollis or abnormal movements) were observed in more than half of the mice. Thus *E. dermatitidis* has a predilection for the mouse brain.

Table 1. Physiological characteristics and GC contents of pathogenic *Exophiala* species and allied genera.

	Suppression of growth at 37°C	Hydrolysis of starch	Splitting of arbutin	Assimilation of KNO_3	Hydrolysis of skim milk	Hydrolysis of gelatin	Hydrolysis of urea	GC content
E. salmonis	+	−	+	+	−	−	+	52·0%
E. pisciphila	+	−	+	+	−	−	+	51·5
E. dermatitidis	−	−	+	−	−	−	+	58·6
*E. jeanselmei**	+	−	−	+	−	−	+	59·5
*E. jeanselmei***	+	−	+	+	−	−	+	59·0
E. moniliae	+	−	+	+	−	−	+	59·0
E. spinifera	−	−	−	+	−	−	+	59·7
H. werneckii	+	−	+	+	+	−	+	54·0
P. verrucosa	−	−	+	+	−	−	+	56·5
P. richardsiae	−	+	+	+	+	+	+	55·5
P. repens	−	+	+	+	+	+	+	51·5
P. parasitica	−	+	+	+	+	+	+	53·4
C. carrionii	−	−	+	+	+	−	+	
C. trichoides	−	−	−	+	−	−	+	
F. pedrosoi	−	−	−	+	−	−	+	
R. atrovirens	+	−	+	+	−	−	+	60·0
R. aquaspersa	+	−	+	+	−	−	+	

E: *Exophiala*, *H*: *Hortaea*, *P*: *Phialophora*, *C*: *Cladosporium*,
R: *Rhinocladiella*

*: A culture derived from the type culture of *T. jeanselmei*.

**: A culture derived from the neotype culture of *P. gougerotii*.

Successively, defense mechanisms of mice against *E. dermatitidis* IMF 4826 were studied using congenitally athymic nude (nu/nu) mice and their heterozygous (nu/+) littermates of BALB/c background (Nishimura and Miyaji 1983). Each mouse was inoculated intravenously with 10^6 yeast cells of the fungus and sacrificed at adequate intervals up to 62 days post inoculation. The number of viable yeast cells in the brains, kidneys and livers of the nu/nu and nu/+ mice was determined. Other internal organs were also examined histopathologically.

The most favourite target organ of culture IMF 4826 was the brain in both groups of mice, followed by the kidney and liver, respectively. The susceptibility of the nu/nu mice to the culture was higher than that of the nu/+ mice.

Colony forming units contained in 100 mg of the brain of the nu/+ mice reached a peak on the 4th day. Thereafter it decreased gradually and from day 40 on, viable cells were never recovered. In the nu/nu mice the maximum colony forming unit also occurred on the 4th day. Thereafter the number of colony forming units remained constant until the end of the experiment (62 days) (figure 19).

The chief histopathological features at the early stages of infection were micro-abscesses (figure 20) and microgranulomas. The microgranulomas were formed in both the nu/+ and nu/nu mice within 2 days. After 8 days, even in the nu/nu mice, almost all of the lesions became granulomatous (figure 21). The microgranulomas in the brain of

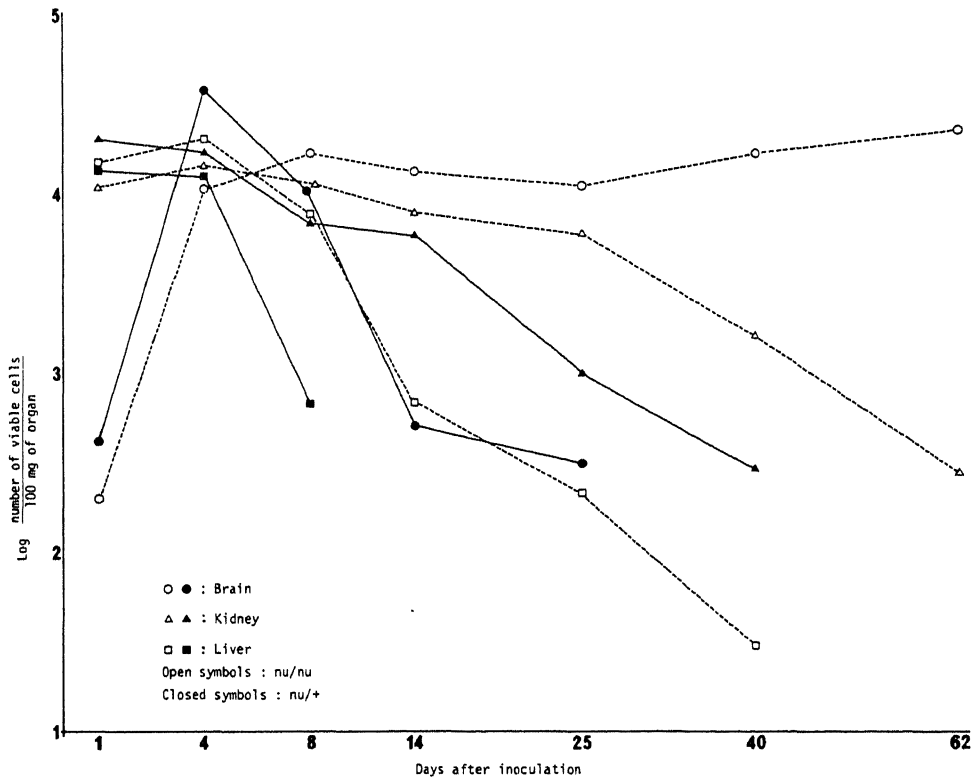


Figure 19. Numbers of viable cells in the organs of nu/+ and nu/nu mice inoculated intravenously with 10^6 yeast cells of *E. dermatitidis* IFM 4826.

the nu/+ mice disappeared after 40 days, while considerable numbers of granulomas were observed in the nu/nu mice until the end of the experiment (62 days).

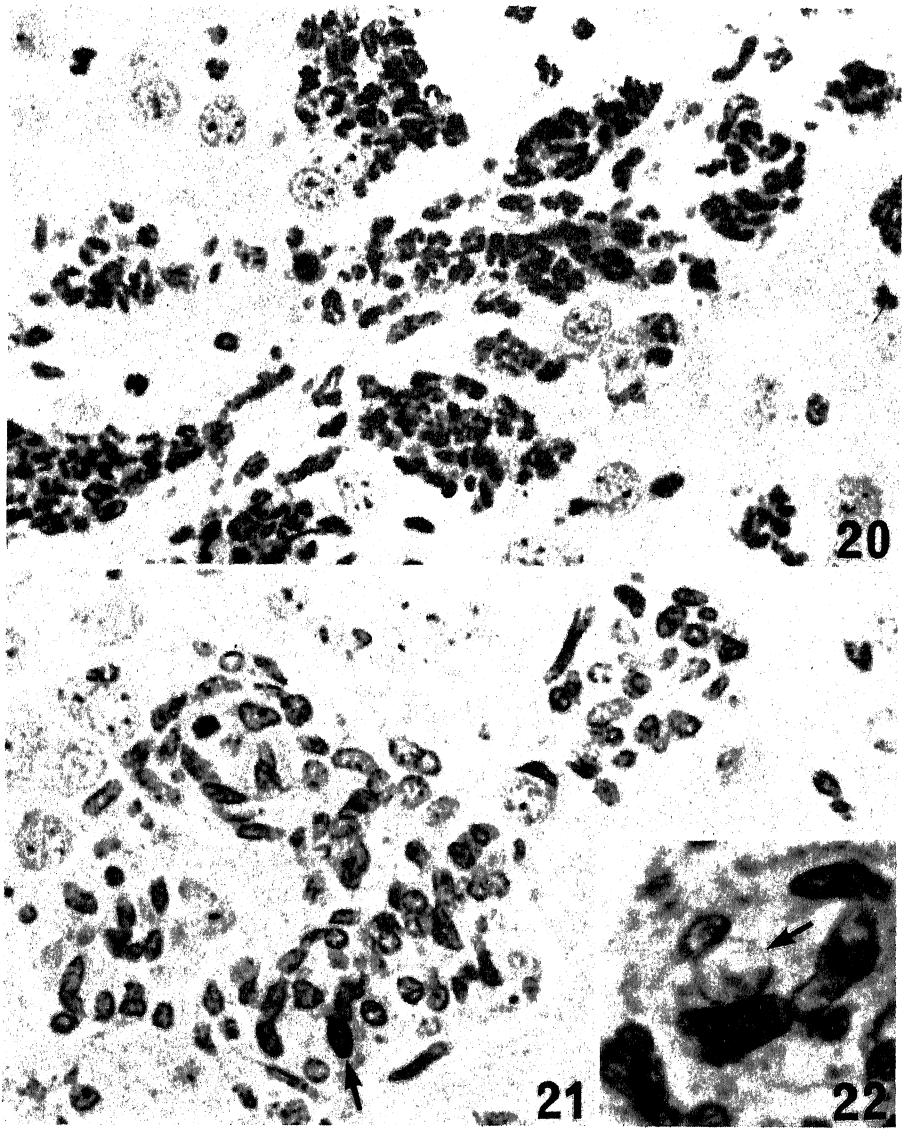
The granuloma formed in the nu/nu mice was morphologically indistinguishable from that formed in the nu/+mice. However, mononuclear cells forming the granulomas in the brain of the nu/nu mice did not kill the yeast cells in them, but suppressed multiplication of the yeast cells. Some yeast cells in the granulomas in the brain were coated with acid mucopolysaccharide (figure 22).

4.2 *Exophiala jeanselmei*

Since 1928, *Exophiala (Torula) jeanselmei* has been known as one of the causative agents of mycetoma (Langeron 1928), while *Phialophora gougerotii* (Young and Ulrich 1953) has been isolated from patients with phaeohyphomycosis since 1953. Because these two fungi are morphologically and biologically similar to each other, various synonyms have been proposed. Emmons (1966) transferred *Torula jeanselmei* to the genus *Phialophora* and insisted that it was different from *P. gougerotii*. However, McGinnis and Padhye (1977) unified these two taxa and proposed a new combination, *Exophiala jeanselmei*, to accommodate them. However, Emmons' point of view concerning the pathogenicity for humans and parasitic forms is still noticeable because the tissue responses and parasitic forms in mycetoma are different from those in phaeohyphomycosis. This might depend upon the difference in virulence among cultures.

The pathogenicity of three cultures isolated as "*jeanselmei*" was compared with three cultures of "*gougerotii*" using ddY male mice. Each mouse was inoculated intravenously with 0·2 ml of a 1% yeast-like cell suspension. 0·2 ml of the cell suspension contained $1{\cdot}25 \times 10^7$ to $8{\cdot}25 \times 10^7$ yeast-like cells. The inoculated mice were observed for 30 days. There were no dead mice in either group until the end of this experiment (30 days). Not all the mice grew weak from infection, and the central nervous syndrome never appeared. They gained weight steadily as if in good health. *E. jeanselmei* is much less virulent to mice than *E. dermatitidis*. As a result of the recovery of the cultures of *P. jeanselmei* from various visceral organs, the two cultures were excluded at the relatively early stages of infection from various visceral organs, while the three cultures derived from *P. gougerotii* were recovered from the brain, heart, liver or kidney until the end of the experiment (table 2).

Histopathologically, microfoci appeared mainly in the liver and lung of both groups of mice, but the cellular responses against the fungi were mild. There was only a slight difference in the histopathological characteristics of the lesions caused by either taxa. Generally, the cellular responses against *P. jeanselmei* were more severe than those of *P. gougerotii* and the inflammatory lesions of the former disappeared at the relatively early stages of infection. Chief histopathological characteristics of the liver of both groups of mice were Kupffer's cells engulfing yeast-like cells, microgranulomatous lesions with or without yeast-like cells and a few purulent ones at the early stages of infection. They lay scattered in the tissue. After the 7th day micro-abscesses disappeared and the number of microgranulomas increased. On the 26th day most of the lesions disappeared. In the lung, small numbers of microfoci of interstitial pneumonia with yeast-like cells sporadically appeared on the 2nd and 4th days. However, on the 7th day almost all the lesions disappeared. There were no budding cells in the liver and lung lesions.



Figures 20–22. **20.** Nu/nu mouse 4 days after inoculation. Polymorphonuclear leucocytes and a few mononuclear cells are induced to hyphae and form microabscesses. PAS (× 400). **21.** Nu/nu mouse 25 days after inoculation. Microgranulomas with a subglobose yeast cells (arrow). PAS (× 400). **22.** Nu/nu mouse 21 days after inoculation. Acid mucopolysaccharide coating yeast cells (arrow) is stained clearly with alcian blue at pH 2·5. Alcian blue-hematoxylin. (× 1000).

We conclude, (i) the virulence of the three cultures each of *P. jeanselmei* and *P. gougerotii* to ddY mice was mild, (ii) these cultures were not neurotropic, (iii) *P. gougerotii* survived longer in the mouse than *P. jeanselmei*, (iv) there were no definite differences in histopathology of the lesions in mice inoculated with the two taxa.

Table 2. Recovery from the brain, lung, liver and kidney of ddY mice inoculated intravenously with 0·2 ml of a 1% cell suspension of *Exophiala jeanselmei*.

		Days after inoculation				
		14	18	22	26	30
E. jeanselmei						
IFM 4857	Brain	*				
	Lung	—				
	Liver	—	—	—	—	
	Kidney	—	—	—	—	
IFM 4860	Brain	—	—			
	Lung	—				
	Liver	—				
	Kidney	—	—			
IFM 4861	Brain	—				
	Lung	—				
	Liver	—				
	Kidney	—	—			
IFM 4862	Brain	—	—	—	—	
	Lung	—	—	—		
	Liver	—	—	—	—	—
	Kidney	—	—	—	—	
IFM 4872	Brain	—	—	—	—	
	Lung	—	—			
	Liver	—	—	—	—	—
	Kidney	—	—			
Yamamoto	Brain	—	—	—	—	—
	Lung	—	—	—	—	
	Liver	—	—	—	—	
	Kidney	—	—	—	—	

* *E. jeanselmei* IFM 4857 was recovered from the brain for only 4 days.

4.3 *Exophiala spinifera*

The pathogenicity of *E. spinifera* was examined using ddY mice inoculated intravenously with 0·2 ml of a 1% cell suspension. Even though the fungus was recovered from the brain and kidney through the 30th day, the inflammatory reactions were mild. Microfoci were observed sporadically in the organs at the early stages of infection. *E. spinifera* was not neurotropic.

We have no data concerning the pathogenicity of *E. moniliae* and *H. werneckii* for mice.

5. The point at issue in the taxonomy

E. dermatitidis was first isolated from a patient with chromomycosis by Kano (1938), who termed it *Hormiscium dermatitidis*. Since then, various synonyms (Carrión 1950;

Borelli 1955; Cooke 1962; Emmons 1966; Schol-Schwarz 1968; Conant *et al* 1971) have been proposed because it has been regarded as a polymorphic fungus. However, the point of issue at present is, which is correct, *Wangiella* or *Exophiala*.

McGinnis (1977) reported that the conidia of *E. dermatitidis* are produced from phialides without collarettes and proposed a new genus *Wangiella*. In the same year de Hoog (de Hoog 1977) insisted that conidia of this species are percurrently and basipetally produced from conidiogenous foci. According to his description the conidiogenesis of this species is both annellidic and phialidic. He took note of the annellidic conidiogenesis and incorporated it into the genus *Exophiala*. According to our observation using scanning electron microscopy, the conidiogenesis of this species is only annellidic. Therefore, *Wangiella* should be rejected.

In *E. jeanselmei* the point at issue is whether or not *P. jeanselmei* and *P. gougerotii* are identical. Emmons (1966) insisted that *P. jeanselmei* is different from *P. gougerotii*. According to him, the former hydrolyzes hypoxanthin and does not assimilate paraffin, while the latter does not hydrolyze hypoxanthin, but assimilates paraffin. Furthermore, he described that the former is a causative agent of mycetoma with granules, while the latter an agent of subcutaneous abscesses and granulomatous lesions with hyphae. According to our data, there are no major differences in the histopathology in mice inoculated with both the taxa. The differences in clinical aspects might occur due to differences in inoculation sites or host defense.

McGinnis and Padhye (1977) reported that these two taxa are morphologically identical and unified them into *E. jeanselmei*. Additionally, they reconfirmed Wang's (1966) observation that the conidial ontogenesis of *Torula (E.) jeanselmei* is annellidic. We also can not distinguish *P. jeanselmei* from *P. gougerotii* morphologically and biologically. The conidial ontogenesis of these fungi is only annellidic. Although there was a small difference in pathogenicity for mice between the two taxa, we agree with McGinnis and Padhye.

Last, we will discuss *H. werneckii*. Horta (1921) described the causative agent of tinea nigra as *Cladosporium werneckii*. Since then, many synonyms have been proposed, confusing the taxonomy of this species. Since von Arx (1970) described it as *Exophiala werneckii* in his book, many mycologists have regarded it as a member of the genus *Exophiala*. Thereafter, Gustafson *et al* (1975) and Cole (1978) found annellations on yeast-like cells of the fungus using transmission and scanning electron microscopy, respectively. McGinnis (1979) also reported that both yeast-like and hyphal conidiogenous cells are annellides, and suggested that the conidiogenesis of the species is only annellidic. Cole (1978) demonstrated a scanning electron micrograph of *E. werneckii*, in which two conidia arose from a short lateral branch. According to him, they occurred in sympodial succession. However, his comment was so brief that it has not been clarified whether or not the fungus has a sympodial anamorph in addition to the annellidic one.

According to our observations by scanning electron microscopy, the conidiogenesis of *H. werneckii* consists of a combination of sympodial and annellidic anamorphs. Its sympodial one is particularly unique. On the basis of these results, we proposed the new genus *Hortaea*, to accommodate *C. werneckii*.

6. Conclusions

(i) The conidial ontogenesis of the pathogenic black yeasts *E. dermatitidis*, *E. jeanselmei*, *E. moniliae* and *E. spinifera* is not pleomorphic, but only annellidic,

(ii) the conidial ontogenesis of *H. werneckii* is both sympodial and annellidic, (iii) *E. dermatitidis* is neurotropic for mice, but *E. jeanselmei* and *E. spinifera* are not, (iv) the GC contents of the *Exophiala* species pathogenic for humans are considerably different from those of *E. salmonis* and *E. pisciphila* which are pathogenic for fish.

References

von Arx J A 1970 *The genera of fungi sporulating in pure culture* 1st edn, J. Cramer, Lehre p. 180

Borelli D 1955 *Sporotrichum gougerotii, Hormiscium dermatitidis, Phialophora jeanselmei: Phialophora gougerotii* (Matruchot, 1910) *comb. n.; Memorias VI Congreso Venezolano de Ciencias Medias* **5** 2945–2971

Butterfield W and Jong S C 1976 Effect of carbon source on conidiogenesis in *Fonsecaea dermatitidis* an agent of chromomycosis; *Mycopathologia* **58** 59–62

Carmichael J W 1966 Cerebral mycetoma of trout due to a *Phialophora*-like fungus; *Sabouraudia* **5** 120–123

Carrión A L 1950 Yeast-like dematiaceous fungi infecting the human skin; *Arch. Dermatol.* **61** 996–1009

Carrión A L and Silva M 1955 Sporotrichosis special reference: a revision of the so-called *Sporotric' um gougerotii*; *Arch. Dermatol. Syph.* **72** 523–534

Cole G T 1978 Conidiogenesis in the black yeasts; *The black and white yeasts*, (Washington D C: Pan American Health Organization Sci. Publ.), No. 356, pp. 66–78

Conant N F, Smith D T, Baker R D, Callaway J L and Martin D S 1971 *Phialophora dermatitidis*; *Manual of clinical mycology* 3rd edn (Philadelphia: W B Saunders) pp. 514–516

Cooke W B 1962 A taxonomic study in the black yeasts; *Mycopathol. Mycol. Appl.* **17** 1–43

Emmons C W 1966 Pathogenic dematiaceous fungi; *Jpn. J. Med. Mycol.* 7 233–245

Emmons C W, Binford C H, Utz J P and Kwon-Chung K L 1977 Chromomycosis, phaeomycotic cyst; *Medical mycology* 3rd edn, (Philadelphia: Lea and Febiger) pp. 386–405, 425–463

Grove S N, Oujezdsky K B and Szaniszlo P J 1973 Budding in the dimorphic fungus *Phialophora dermatitidis*; *J. Bacteriol.* **115** 323–329

Gustafson R A, Hardcastle R V and Szaniszlo P J 1975 Budding in the dimorphic fungus *Cladosporium werneckii*; *Mycologia* **67** 942–951

de Hoog G S 1977 *Rhinocladiella* and allied genera; *CBS studies in mycology No. 15 The black yeasts and allied hyphomycetes*, pp. 1–132

Horta P 1921 Sobre um caso de tinha preta e um novo cogumelo (*Cladosporium werneckii*); *Rev. Med. Cirurgica do Brasil* **29** 267–274

Kano K 1938 Über die Chromoblastomykose durch einen noch nicht als pathogen beschriebenen Pilz: *Hormiscium dermatitidis* n. sp.; *Arch. Dermatol. Syph.* **176** 282–294

Langeron M 1928 Mycetome a *Torula jeanselmei* Langeron 1928 nouveau type de mycétome à grains noirs; *Ann. Parasitol. Hum. Comp.* **6** 385–390

McGinnis M R 1977 *Exophiala spinifera*, a new combination for *Phialophora spinifera*; *Mycotaxon* **5** 337–340

McGinnis M R and Padhye A A 1977 *Exophiala jeanselmei*, a new combination for *Phialophora jeanselmei*; *Mycotaxon* **5** 341–352

McGinnis M R 1977 *Wangiella*, a new genus to accommodate *Hormiscium dermatitidis*; *Mycotaxon* **5** 353–363

McGinnis M R 1978 Human pathogenic species of *Exophiala, Phialophora*, and *Wangiella*; *The black and white yeasts*, (Washington D C: Pan American Health Organization, Sci. Publ) No 356, pp. 37–59

McGinnis M R 1979 Taxonomy of *Exophiala werneckii* and its relationship to *Microsporum mansonii*; *Sabouraudia* **17** 145–154

McGinnis M R, Sorrell D F, Miller R L and Kaminski G W 1981 Subcutaneous phaeohyphomycosis caused by *Exophiala moniliae*; *Mycopathologia* **73** 69–72

Nielsen Jr H S and Conant N F 1968 A new human pathogenic *Phialophora*; *Sabouraudia* **6** 228–231

Nishimura K and Miyaji M 1982 Studies on a saprophyte of *Exophiala dermatitidis* isolated from a humidifier; *Mycopathologia* **77** 173–181

Nishimura K and Miyaji M 1983 Defense mechanisms of mice against *Exophiala dermatitidis* infection; *Mycopathologia* **81** 9–21

Nishimura K and Miyaji M 1983 Studies on the phylogenesis of pathogenic 'black yeasts'; *Mycopathologia* **81** 135–144

Nishimura K and Miyaji M 1984 *Hortaea*, a new genus to accommodate *Cladosporium werneckii*; *Jpn. J. Med. Mycol.* **25** 139–146

Oujezdsky K B, Grove S N and Szaniszlo P J 1973 Morphological and structural changes during the yeast-to-mould conversion of *Phialophora dermatitidis*; *J. Bacteriol.* **113** 468–477

Padhye A A 1978 Comparative study of *Phialophora jeanselmei* and *P. gougerotii* by morphological, biochemical, and immunological methods; *The black and white yeasts.* (Washington D C: Pan American Health Organization, Sci. Publ.) No 356, pp. 60–65

Schol-Schwarz M B 1968 *Rhinocladiella*, its synonym *Fonsecaea* and its relation to *Phialophora*; *Antonie van Leeuwenhoek* **34** 119–152

Young J M and Ulrich E 1953 Sporotrichosis produced by *Sporotrichum gougerotii*; *A. M. A. Arch. Derm Syph.* **67** 44–52

Wang C J K 1966 Annellophores in *Torula jeanselmei*; *Mycologia* **58** 614–621

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 453–463.
© Printed in India.

Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals

EDOUARD DROUHET, CLAUDE de BIEVRE and
SEEMA WAHAB*
Institut Pasteur, Unité de Mycologie, 75015 Paris, France

Abstract. Four species of *Drechslera* usually soil saprophytes or plant parasites were recognized over the last few years as agents of deep mycoses with subcutaneous, osteolytic, endocardic and pulmonary localizations as well as superficial mycoses of cornea and skin. The key of the potentially pathogenic fungi *Drechslera longirostrata* (agent of an endocarditis and spondylodiscitis), *Drechslera hawaiensis* (agent of a pulmonary mycosis) *D. rostrata* and *D. spicifera* (agents of several cases of phaeohyphomycoses) is described. The sensitivity of these species to actual systemic antifungal agents (amphotericin B, ketoconazole, itraconazole) is reported.

Keywords. *Drechslera* species; mycoses; antifungal agents; amphotericin B; ketoconazole; itraconazole.

1. Introduction

A great variety of fungi, soil saprophytes or plant parasites usually considered as non-pathogenic to man may become pathogenic in humans and animals when the host conditions are favourable; these fungi are called opportunistic (Drouhet 1972) and may produce superficial or deep mycoses, sometimes very severe as we observe with *Drechslera longirostrata* (Subramanian) *comb. nova*; this fungus usually isolated from dead leaves (Subramanian 1956; Subramanian and Jain 1966) rice and sorghum (Nath *et al* 1970) was recognized recently (Drouhet *et al* 1982) for the first time in literature as responsible of an endocarditis and spondylodiscitis in a young woman operated in Martinica for a cardiac valve prosthesis.

Three other species of *Drechslera* chiefly Graminae pathogens or soil saprophytes were recognized during the last few years as responsible for various human and animal mycoses called phaeohyphomycoses (Ajello *et al* 1980) due to the dematiaceous characters of the filaments invading the deep tissues: *Drechslera hawaiensis* (Fuste *et al* 1973; Koenig *et al* 1984; Krachmer *et al* 1978; McAleer *et al* 1981; Young *et al* 1978), *D. rostrata* (Ajello *et al* 1980; Forster *et al* 1975; McKenzie and Connole 1977) and *D. spicifera* (Bridger *et al* 1960; Estes *et al* 1977; Kaplan *et al* 1975; Patton 1977; Sobol *et al* 1984; Zapater *et al* 1975).

In honour of Prof. C V Subramanian, whose work consecrated to the Hyphomycetes (Subramanian 1956; Subramanian and Jain 1966; Subramanian 1983) is internationally unanimously appreciated*, we relate in this contribution the principal mycological and pathological characters of the *Drechslera* opportunistic species, pathogenic for

* Post-doctoral fellow under Indo-French Scientific Cooperation

* Prof. C V Subramanian was elected President of the International Mycological Association and of the 3rd International Congress of Mycology held in Tokyo from 28th August– 3rd September 1983.

man and animals, as well their sensitivity to antifungal agents in view of an effective chemotherapy.

2. Mycological aspects

Drechslera Ito, Proc. Imp. Acad. Jpn 6: 355, 1930 nom. cons

2.1 *General characters*

Taxonomic studies by Ito (1930) and Shoemaker (1957), showed that the genus *Helminthosporium* was a heterogeneous assemblage of species. This realization led to the creation of 2 new genera—*Drechslera* Ito (1930) and *Bipolaris* Shoemaker (1959) to accommodate the anomalous species.

The genus contains 40 to 45 species (Ellis 1971, 1976). Many of the species are cosmopolitan but are more frequent in tropical or subtropical countries. Most of the known host plants belong to the family Graminaceae. *Drechslera* may cause leaf spot, seedling blights or root rots. Several species are opportunistic and cause diseases in man and animals.

On malt agar the colonies are fast to mild growing, velvety to cottony, grey, brown or blackish. Reverse is dark brown to black, hyphae are hyalin or pigmented. Conidiophores are single or in small groups, generally unbranched, and geniculated due to sympodial elongation; spores are produced through a conspicuous pore (poroconidia) fusiform to ellipsoidal, pale to dark brown, smooth walled with several pseudoseptates (phragmospores) sometimes with end cells pale coloured.

The relationship of *Drechslera* with other allied genera and connexious anamorph-teleomorph are discussed by Lutrell (1977). On the basis of the spore germination some authors maintain *Bipolaris* Shoemaker (1959) as a separate genus, the conidia of the *Drechslera sensu stricto* germinate from any cell and those of *Bipolaris* by the end cells. Some species showing a protuberant hilum in conidia, like *Drechslera rostrata*, are placed in the new genus *Exserohilum* Leonard and Suggs (1974) but we do not follow this opinion.

2.2 *Key to the potentially pathogenic species*

All these species grow at 37°C, which is an obligatory condition for human and animal pathogenicity.

Drechslera longirostrata Subramanian

Conidiophores thick-walled, single or in groups of 2–5 bearing 2–6 poroconidia at their tip. Poroconidia have numerous septa (up to 24). The basal and rarely apical septum are darker. Conidia with prominent hilum are mostly stout, sometimes abruptly bent.

According to the medium the maximum length varies (as very often with other species) from 310 to 434 μm and the width from 12 to 20 μm. This species is partly distinguished from the closely related *Drechslera rostrata* on the basis of the length and the arrangement of conidia and top darker septum in *Drechslera longirostrata*. Groups of conidia, provided with a dark basal septum, give a palm tree like appearance. On the contrary in *Drechslera rostrata*, conidia provided with a dark basal and top septum are

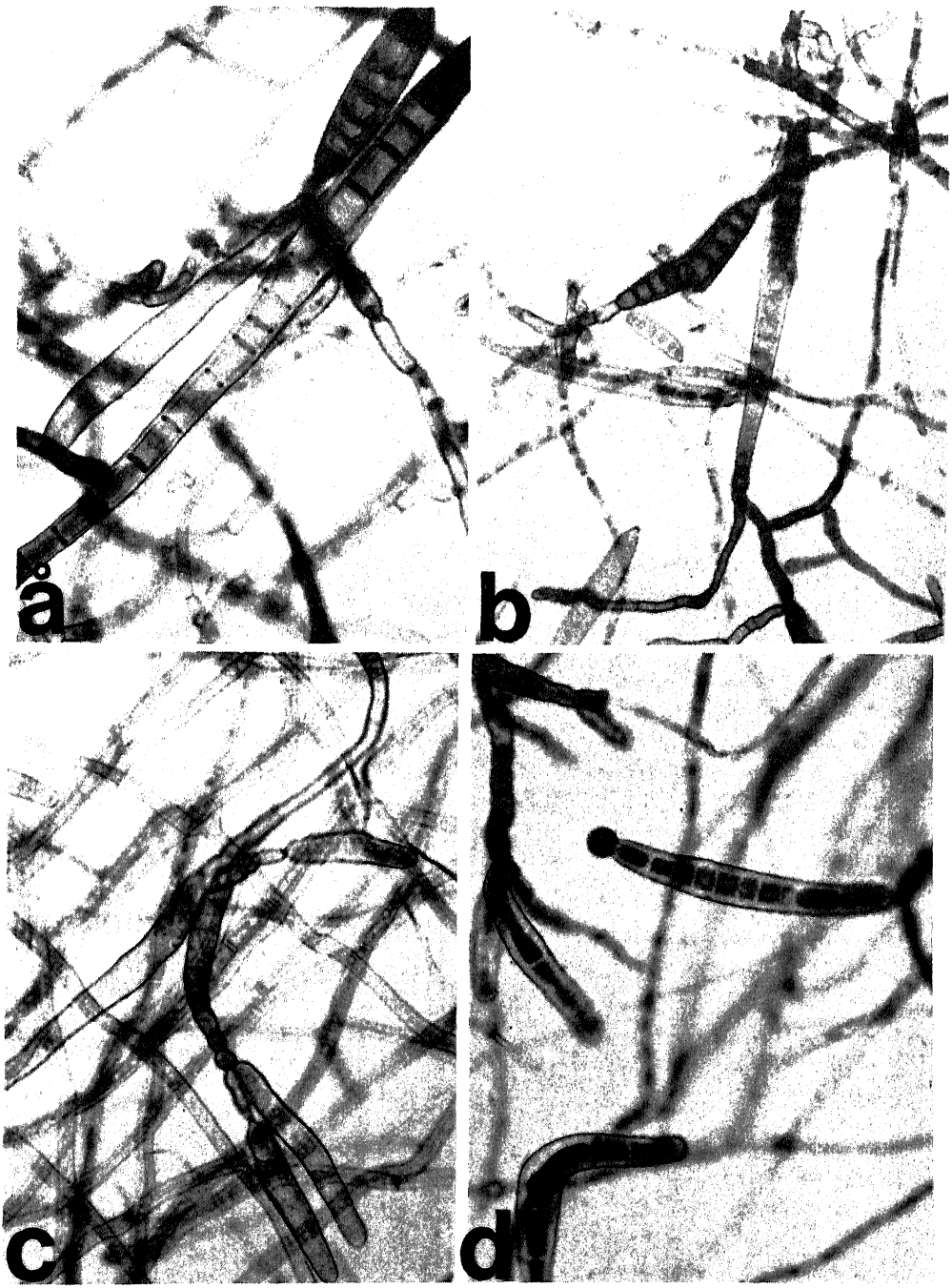


Figure 1. Morphology of *Drechslera longirostrata* strain IP 1229–80 from human endocarditis. Malt extract agar (a,b), Sabouraud glucose agar (c,d). **a.** Multiseptated macroconidia (17 septa) (obj. × 100 HI oc × 10) **b.** Macroconidia up to 20 septa (obj. × 40 oc × 10). **c,d.** Macroconidia (obj. × 40 oc × 10).

arranged in an alternate fashion. Figure 1 illustrates the microscopic morphology of the strain isolated from mycotic endocarditis and osteoarthritis.

Drechslera rostrata (Drechsler) Richardson and Fraser. *Exserohilum rostratum*—(Drechsler) Leonard and Suggs. Teleomorph: *Setospheria rostrata* (Drechsler) Leonard (1976).
Conidiophores single or in small clusters, generally flexuous or geniculate, dark brown, up to 200 μ long and 6–8 μ thick. Poroconidia stout, slightly curved to obclavate with 6 to 10 dark septa, end cells less pigmented, 40 to 180 μ long and 14 to 22 μ thick, with a prominent hilum.

Figure 2 illustrates the microscopic morphology of some of the strains studied:

IMI 76563—ex soil, India, coll. R. Y. Roy, 1959 (in the catalogue of the Commonwealth Mycological Institute under *D. halodes* which is now considered conspecific with *D. rostrata*).

IMI 136668—ex leaf of *Sorghum* sp., Cairo, UAR, Coll. O. Abdel-Azim—CMI 1968.

IMI 197559—ex *Zea mays* stalk, USA, 1972, coll. T A Kucharek (Luttrell 8868)—K J Leonard (mating type *a*).

IMI 197560—ex Hay 3 × IMI 76563, USA, K Leonard (S_1A_3 – mating type *A*).

Drechslera hawaiensis (Bugnicourt) Subramanian and Jain ex Ellis 1966. Perfect state: *Cochliobolus hawaiensis*. In 1966 Subramanian and Jain emended the genus *Drechslera* and transferred the species *Helminthosporium hawaiensis* to the genus *Drechslera*.

Conidiophores solitary flexuous to geniculate with several septa, brown coloured up to 100 to 120 μm long and 2–7 μm thick. Poroconidia ellipsoidal, oblong to cylindrical with rounded ends, pale to dark brown, mostly 5 septate, but varying from 3 to 7 septate. In some strain length varies from 12 to 37 μm. Thickness varies from 5 to 11 μm; 5 septate poroconidia are mostly 9 × 25 μ. Figures 3 a, b, c illustrate the microscopic morphology of the strain isolated from the case reported by Koenig *et al* (1984).

Drechslera spicifera Nelson. Teleomorph: *Cochliobolus spicifer* Nelson 1964.
Conidiophores as in other species arise singly or in small clusters, repeatedly geniculate, with conspicuous scars giving a typical appearance, mid to dark brown, up to 300 μ long and 4 to 9 μ thick. Poroconidia are oblong or cylindrical with rounded ends generally golden brown in colour, with 3 septa length varies from 20 to 40 μm and thickness from 9 to 14 μm. Figure 3d illustrates the microscopic morphology of a human strain of *D. spicifera*.

3. Parasitic and saprophytic life of *Drechslera* species potentially pathogens for man and animals

3.1 *Drechslera longirostrata*

D. longirostrata was isolated by Subramanian (1956) from dead leaves of *Borassus flabellifer* and by Nath *et al* (1970) as seed-borne in rice and sorghum, as well as other seeds (wheat, pearl, millet). Experimental infections carried out at 2 stages of growth of rice and sorghum proved their plant pathogenicity but their role in growing crops remains to be established.

The first human case of mycosis due to *D. longirostrata* was reported by Drouhet *et*

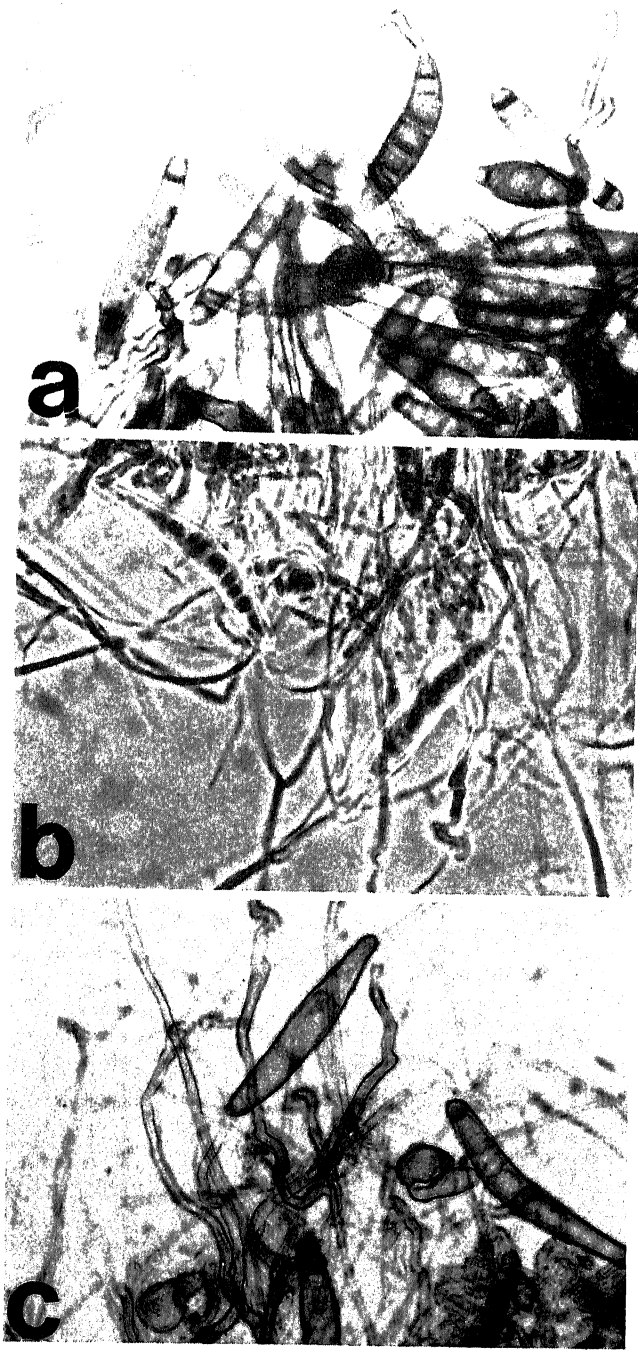


Figure 2. Macroconidia of *Drechslera rostrata*. **a.** strain IP 1260 CMI 197560 **b, c.** strain IP 1263 CMI 76563 (obj. × 40 oc × 10).

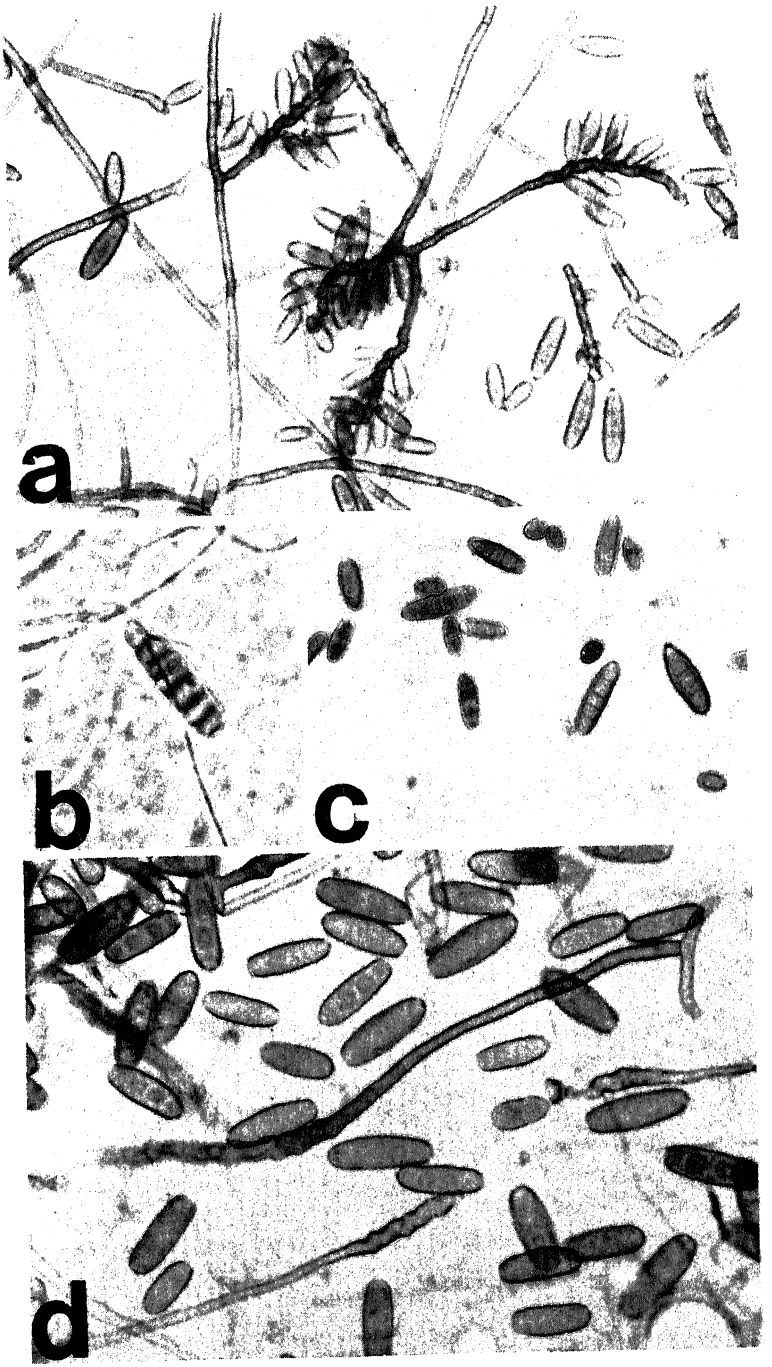


Figure 3 **a.** Macroconidia and conidiophores of *Drechslera hawaiensis* strain IP 1513–83 from a human pulmonary mycosis (obj. 40 oc × 10). **b.** Obj. × 100 CMI oc × 10. **c.** Obj. 40 oc × 10. **d.** Macroconidia and conidiophores of *D. spicifera* (Obj. × 100 oc × 10).

al (1982); the patient a young woman, 20 year old, operated at Fort de France (Martinica) with valvular prosthesis for a congenital heart defect, developed in the following 3 months a mycotic endocarditis diagnosed when she was reoperated in Paris. At surgery there was an aortic valvular vegetation in which hyphal elements were seen microscopically and from which *D. longirostrata* was isolated and identified. In spite of amphotericin B treatment followed by ketoconazole treatment, vertebral arthritis due to invasion of the fungus localized first at the intervertebral disc of 2 lumbar vertebral was extended to 3 lumbar segments. Only combination of the 2 antifungal agents, amphotericin B and ketoconazole, permitted the complete cure of this severe case.

3.2 *Drechslera rostrata*

This species is cosmopolitan and common on Graminae and irregularly isolated from soil (Ellis 1971).

Jones (1975) incriminated *D. rostrata* as an agent of oculomycosis.

Ajello *et al* (1980) described this species as an agent of phaeohyphomycosis in a 12 year-old black boy from Bakersfield (California); the severe chronic and progressive osteolytic infection in the foot of this boy was cured after six months of amphotericin B treatment. An experimental infection was obtained in some mice inoculated by intraperitoneally: a lesion noted in the liver of a mouse was found to contain dematiaceous mycelium. *D. rostrata* was observed as an agent of mycoses in Australian boyines; Pritchard and Chick (1977) described multiple mycetoma in a cow with multiple lesions of the skin, nasal mucosa and lymph nodes and mycelial elements organized into granules; McKenzie and Connole (1977) observed also mycotic nasal granuloma due to *D. rostrata* in cattle.

3.3 *Drechslera hawaiensis*

This species described by the French mycologist Bugnicourt in 1955 was first isolated from rice in Hawai and later from several other plants in many countries but particularly in the tropical and subtropical areas.

D. hawaiensis was incriminated as the etiologic agent of a fatal case of meningoencephalitis in Memphis, Tennessee (Fuste *et al* 1973); the victim was a 31 year old woman with an underlying lymphocytic lymphosarcoma. Numerous hyphae, (2–4·2 μm dia.) bizarrely shaped and swollen fungal cells were observed during autopsy in the brain. Experimental infection in guinea pigs and mice were obtained with the human isolate as well as with plant isolates of *D. hawaiensis*.

In France, *D. hawaiensis* has been isolated by Koenig *et al* (1984) from a pulmonary allergic bronchocele in a 12 year old boy who lived in Tahiti. The septate, dematiaceous hyphae were observed in the pulmonary excised lesion. Eosinophilic infiltrative lesions accompanied the pulmonary granuloma; the child is still under ketoconazole treatment. A nasal obstruction with bone erosion caused by *D. hawaiensis* in a 15 year old boy was reported by Young *et al* (1978). Several corneal ulcers due to a *Helminthosporium* sp. presumed to be *D. hawaiensis* according to de Hoog (1983) were reported by Krachmer *et al* (1978). In animals granulomatous proliferations of the nasal mucosa were described in cattle by Roberts and co-workers as due to

Helminthosporium anomalum. Gilman and Abbott; according to de Hoog the fungus of this case is also recognized as *D. hawaiensis.*

3.4 *Drechslera spicifera* (Bainer)

Plant parasites, isolates from textiles, soil and air in all parts of the world, especially in the tropical and subtropical areas were identified as *D. spicifera* (Ellis 1971).

In man, Zapater *et al* (1975) and Forster *et al* (1975) reported the first cases of corneal ulcers due to *D. spicifera.*

A primary cutaneous phaeohyphomycosis in a 5 year old leukemic boy was reported by Estes *et al* (1977). Hyphae and swollen hyphal cells resembling chlamydospores were observed in the body specimens. Resolution of the infection occurred following excisional biopsy and systemic amphotericin B therapy. Two cases of chronic pneumonia caused by a *Helminthosporium* sp that retrospectively seems to be close to *D. spicifera* (de Hoog 1983) were reported by Dolan *et al* (1970).

In animals, phaeohyphomycoses due to *D. spicifera* was reported by Kaplan *et al* (1975) in a young horse from Virginia, and by Muller *et al* (1975) in a cat. Clinically the horse had multiple black, pustulous and papular cutaneous plaques, 1–3 cm in diameter; within the tissues septate hyphae measuring 5 to 35 μm in diameter were observed. The cat also showed chronic granulomatous skin lesions as well as the cat mycetoma reported by Bridges and Beasley (1960). A maduromycotic type mycetoma was also reported in an aged mare due to a fungus close to *D. spicifera* (Schauffer 1972), in a dog (Hall 1965) and in a cow (Patton 1977). Recently Sobol *et al* (1984) reported a new case of phaeohyphomycosis of the maxilloethmoid sinus caused by *Drechslera spicifera.*

3.5 *Sensitivity of Drechslera pathogenic strains to systemic antifungal agents*

The four species of *Drechslera*, potentially pathogenic for humans and animals *D. longirostrata, D. rostrata, D. hawaiensis* and *D. spicifera* are agents of deep mycoses with subcutaneous, osteoarticular, pulmonar or endocardic localisations; their severity of infection and chronicity necessitate systemic chemotherapy by intravenous amphotericin B and oral ketoconazole combined with surgical excision; for superficial cutaneous or ocular localisations, topical treatment (iodide, amphotericin B, pimaricin) showed some limited effects. The infection severity of the first reported case of *D. longirostrata* infection in man (Drouhet *et al* 1982), where a spondylodiscitis extended to 3 lumbar vertebrae as a complication of endocarditis from a valve prosthesis infection, was cured only after combined administration of i.v. amphotericin B and oral ketoconazole; both drugs have been proved unsuccessful when given alone. The minimal inhibitory concentration (MIC) of each individual tested antifungal agent in liquid casitone medium was 1 μg/ml, while complete inhibition of the growth was recorded at 0·12 μg amphotericin B and 0·25 μg/ml ketoconazole, showing synergism which explains the excellent therapeutic effect when the two antifungal agents were used concomitantly. The strains of *D. hawaiensis, D. spicifera* and *D. rostrata* reported from different cases by Young *et al* (1970), Estes *et al* (1977) and Ajello *et al* (1980) were found responsible for severe subcutaneous and osteolytic phoeohyphomycoses and showed their sensitivity to amphotericin B. Their prolonged treatment with this drug associated with surgery permitted complete cure but with sequelae. Among the new

Table 1. MIC of ketoconazole and itraconazole against *Drechslera* sp after 24 and 48 hr using casitone and YNB liquid media, employing microtiter technique.

Strain (Nber)	Ketoconazole (μg/ml)				Itraconazole (μg/ml)			
	Casitone		YNB		Casitone		YNB	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
1	0·39	0·39	3·12	25	0·18	0·18	0·18	0·18
2	0·39	1·56	6·25	12·5	0·18	1·56	6·25	6·25
3	0·39	0·78	–	12·5	0·39	0·39	0·18	0·18
4	1·56	3·125	3·125	50	0·18	0·78	0·18	0·39
5	1·56	1·56	6·25	25	0·18	0·18	0·18	0·39

Concentrations tried: 100, 50, 25, 12·5, 6·25, 3·125, 1·56, 0·78, 0·39 and 0·18 μg/ml.
Media: Casitone complex medium (Drouhet and Dupont 1983)
YNB (Difco) + asparagine 1·5 g/l + glucose 10 g/l
Strains: 1: *D. longirostrata* IP 1229–80, 2: *D. rostrata* IP 1262–81 CMI 197559
3: *D. rostrata* IP 1263–81 CMI 75563, 4: *D. rostrata* IP 1260–80 CMI 197568
5: *D. hawaiensis* IP 1503–83

imidazole derivatives two systemic antifungal agents, the ketoconazole (Drouhet and Dupont 1983) and itraconazole (R 51211) (Van Cutsem *et al* 1983) are promising drugs in systemic mycoses. The sensitivity of several strains of *Drechslera* species studied in our laboratory showed that these drugs are effective in vitro against these potentially pathogenic fungi for human and animals and may be used in vivo for the treatment of their mycotic infection.

4. Conclusions

Among the great variety of fungi, soil saprophytes or plant parasites considered usually as non-pathogenic for human and animals, four species of *Drechslera* were recognized in recent years as agents of deep mycoses with subcutaneous, osteolytic, endocardic and pulmonary localizations as well as superficial mycoses of cornea and skin. The potential pathogenicity of species of *Drechslera longirostrata* (Subramanian) Nath, *D. rostrata* (Drechsler) Richardon and Fraser, *D. hawaiensis* (Bugnicourt) Subramanian and Jain and *D. spicifera* Nelson, is described as well the pathological manifestations in humans and animals have also been reviewed. *D. longirostrata* reported for the first time as an agent of endocarditis followed by vertebral localizations in a young women submitted to heart surgery. A pulmonary mycosis was also observed in France in a boy arriving from Tahiti. The other cases reported in literature are particularly from tropical and subtropical countries.

The above mentioned four species are sensitive to systemic antifungal agents (amphotericin B, ketoconazole and itraconazole) but the chemotherapy necessitates in some severe cases a prolonged treatment with combined surgery.

Acknowledgements

The authors express their thanks to Dr Phyllis Stockdale and Dr A Sivanesan (Commonwealth Mycological Institute Kew, UK) for help in identification of the strain

of *D. longirostrata* and the cultures of various *Drechslera* species sent for comparison. They acknowledge Lvce Improvisi, O Ronin, A Pietfroid and Marie-Claire Thibovt for their excellent technical assistance and Dr J Van Cutsem (Jansen Pharmaceutica, Beerse, Belgium) for Ketoconazole and itraconazole samples.

References

Ajello L, Iger M, Wybel R and Vigil F J 1980 *Drechslera rostrata* as an agent of phaeohyphomycosis; *Mycologia* **72** 1094–1102

Bridges C H and Bealsley J N 1960 Maduromycotic mycetomes in animals *Brachycladium spiciferum* as an etiologic agent; *J. Am. Vet. Med. Assoc.* **137** 192–201

Dolan C T, Weed L A and Dines D E 1970 Bronchopulmonary helminthosporiosis; *Am. J. Clin. Pathol.* **53** 235–242

Drechsler C 1923 Some graminicolous species of *Helminthosporium*; *J. Agric. Res.* **24** 641–740

Drouhet E 1972 Champignons opportunistes et mycoses iatrogènes; *Bull. Inst. Pasteur (Paris)* **70** 391–464

Drouhet E, Guilmet D, Kouvalchouk J F, Chapman A, Ziza J M, Laudet J and Brodaty D 1982 Premier cas humain de mycose à *Drechslera longirostrata.* Spondylodiscite compliquant une endocardite sur prothèse. Traitement par l'association ketoconazole-amphotéricine **B**; *Nouv. Presse Méd.* **11** 3631–3635

Drouhet E and Dupont B 1983 Laboratory and clinical assessment of ketoconazole in deep seated mycoses; *Am. J. Med.* **B74** (Suppl.) 30–47

Ellis M B 1971 *Dematiaceous Hyphomycetes* (Kew, Surrey, England: Commonwealth Mycological Institute) 608 p

Ellis M B 1976 *More dematiaceous Hyphomycetes* (Kew, Surrey, England: Commonwealth Mycological Institute) 507 p

Estes S , Merz W G and Maxwell L G 1977 Primary cutaneous phaeohyphomycosis caused by *Drechslera spicifera*; *Arch. Dermatol.* **113** 813–815

Forster R K, Rebell G and Wilson L A 1975 Dematiaceous fungal keratitis; *Br. J. Ophthalmol.* **59** 372–376

Fuste F J, Ajello L, Threlkeld R and Henry J E Jr 1973 *Drechslera hawaiensis*: causative agent of a fatal meningoencephalitis; *Sabouraudia* **53** 233–242

Hall J E 1965 Multiple maduromycotic mycetomas in a colt caused by *Helminthosporium*; *Southwest Vet.* **18** 233–234

Hoog G S de 1983 On the potentially pathogenic dematiaceous hyphomycetes. In *Fungi pathogenic for humans and animals part A.* (ed.) D H Howard (New York: Marcel Dekker) pp. 149–216

Ito S 1930 On some new ascigerous stages of the species of Helminthosporium parasitic on cereals; *Proc. Imp. Acad. (Tokyo)* **37** 352–355

Jones B R 1975 Principles in the management of oculomycosis; *Trans. Am. Acad. Ophthalmol. Otolaryngol.* **79** 15–53

Kaplan W, Chandler F W, Ajello L, Gauthier R, Higgins R and Cayouette P 1975 Equine phaeohyphomycosis caused by *Drechslera spicifera*; *Can. Vet. J.* **16** 205–208

Koenig H, Warter A, de Bievre C, Waller J, Weitzenblum E and Morand G 1984 Mycose pulmonaire à *Drechslera hawaiensis*; *Bull. Soc. Fr. Mycol. Med.* **13** 373–376

Krachmer J H, Anderson R L, Binder P S, Rowsey J J and Meck E S 1978 Helminthosporium corneal ulcers; *Am. J. Ophthalmol.* **85** 666–670

Leonard K J and Suggs E G 1974 *Setospheria prolata*, the ascigerous state of *Exserohilum prolatum*; *Mycologia* **66** 281–297

Leonard K J 1976 Synonymy of *Exserohilum halodes* with *E. rostratum* and induction of the ascigerous state *Setospheria rostrata*; *Mycologia* **68** 402–411

Luttrell E S 1977 Correlation between conidial states and ascigenous state characteristic in Pyronema, Cochliobolus and Setospheria; *Rev. Mycol.* **41** 271–279

McAleer R, Kroenert D B, Edler J L and Froudist J H 1981 Allergic bronchopulmonary disease caused by *Curvularia lunata* and *Drechslera hawaiensis*; *Thorax* **36** 338–344

McKenzie R A and Connole M D 1977 Mycotic nasal granuloma in cattle; *Aust. Vet. J.* **53** 260–270

Muller G H, Kaplan W, Ajello L and Padhye A A 1975 Phaeohyphomycosis caused by *Drechslera spicifera* in a cat; *J. Am. Vet. Med. Assoc.* **166** 150–154

Nath R, Neergaard P and Mathur S B 1970 *Drechslera longirostrata* (Subram.) comb *nov* in seeds of rice and sorghum; *Proc. Int. Seed. Test. Assoc.* **35** 145–150

Pritchard D and Chick B F 1977 Mycotic mycetoma due to *Drechslera rostrata* infection in a cow; *Aust. Vet.* **53** 241–244

Patton C S 1977 *Helminthosporium spiciferum* as the cause of dermal and nasal maduromycosis in a cow; *Cornell. Vet.* **67** 236–244

Schauffer A F 1972 Maduromycotic mycetoma in an aged mare; *J. Am. Vet. Med. Assoc.* **160** 998–1000

Shoemaker R A 1959 Nomenclature of *Drechslera* and *Bipolaris*, parasites segregated from *Helminthosporium*; *Can. J. Bot.* **37** 879–887

Subramanian C V 1956 Hyphomycetes II; *J. Indian Bot. Soc.* **35** 446–494

Subramanian C V and Jain B L 1966 A revision of some graminicolous *Helminthosporia*; *Curr. Sci.* **35** 352–355

Subramanian C V 1983 *Hyphomycetes. Taxonomy and biology* (London: Academic Press) 502 pp

Sobol S M, Love R G, Stutman H R and Pysher T Y 1984 Phaeohyphomycosis of the maxilloethmoid sinus caused by *Drechslera spicifera*: a new fungal pathogen; *Laryngoscope* **94** 620–627

Van Cutsem J, Vangerven F and Zaman R 1983 Pharmacological and preclinical results with a new oral and topical broad spectum antifungal R 51211. *Proc. 13th Int. Congress of Chemotherapy* Vienna, August 28–Sept. 2, Abstract.

Young C N, Swart G and Ackerman D 1978 Nasal obstruction and bone erosion caused by *Drechslera hawaiensis*; *J. Laryngol. Otol.* **92** 137–143

Zapater R C, Albesi E J and Garcia G H 1975 Mycotic keratitis by *Drechslera spicifera*; *Sabouraudia* **13** 295–298

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 465–474.
© Printed in India.

India's changing agriculture: Its management, genetic vulnerability and diversification

H K JAIN

International Service for National Agricultural Research, P.O. Box 93375, 2509 AJ, The Hague, Netherlands

Abstract. The green revolution is here to stay. It must now be taken to its next logical step which is one of closing the continuing yield gaps. The present production advance is based on sound policy decisions, more of which will be needed. Its fundamental basis is the creation of high genetic potentials of crop yields and of new agronomic environments through the use of modern farm inputs for the expression of these potentials on farmers fields. The enhancement of this expression calls for the development of a new management infrastructure to provide a wide range of services to millions of our small and marginal farmers. India's changing agriculture also brings in its wake new problems. One of the more important of these relates to its increasing genetic uniformity. This makes our agriculture more vulnerable to diseases and pests. A major programme of genetic diversification and conservation of genetic resources is suggested to overcome this problem.

Keywords. Indian agriculture; farming systems; genetic potentials; breeding approaches.

1. Introduction

Following a five thousand year history of evolution in a traditional framework, India's agriculture is entering a new phase of scientific transformation. The production of more than 151 million tonnes of food grains this year (1983–84), showing an increase of 17% over the previous year, creates a new record of accelerated growth which has few parallels in the world. Above all, it must confound those critics who continue to find fault with the green revolution, for one reason or the other. First, it was the so-called disparity created by it. Some economists kept arguing that the new agricultural technology had benefitted mostly the large farmers and that it was not very relevant to the small farmers. The argument was patently wrong for it is obvious that Punjab's average wheat and rice yields of three tonnes per hectare would not have been possible, if only the large farmers had increased their production. Nor would Uttar Pradesh be producing more than 15 million tonnes of wheat, if the small farmers in that state had not taken advantage of the new high yielding varieties. More important, West Bengal with its very small land holdings would not be producing so much wheat and rice as it does now if the new agricultural technology was not largely scale neutral. Then, followed the argument about nutrition. The argument was that the new technology of cereal production had led to a decline in pulses so that the nutritional value of the diet had suffered, because of a protein deficit. While admittedly, there has been some diversion of land from pulses like Bengal gram to wheat, a recent analysis carried out by the International Institute for Semi Arid Tropics at Hyderabad shows that the increased availability of cereal grains with the advent of the high yielding varieties programme has helped to increase the protein content of the Indian diet. Also, of course, the new technology of pulses which is now being generated using some of the concepts that have proved so rewarding in the case of cereals should ensure that Indian

agriculture will see a more balanced growth in the coming years. Already, the production of pulses and oilseeds is beginning to see a sustained increase.

More recently, the argument has been that the production advance of the 1970's could not be sustained. The beginning of the eighties saw some particularly bad years in terms of wide spread drought and this was bound to have an adverse effect on agriculture. Nowhere in the world agriculture is immune to weather influences, be it India, Australia or the USA. The more important requirement is that the fluctuations in production should not be great and even in a bad year, the country should be able to maintain a reasonable level of production. Indian agriculture is now beginning to reach this state of maturity. While the 1979–80 drought saw the production of food grains showing a sharp decline with a loss of nearly 18 million tonnes, the equally serious drought of 1981–82 had a much smaller impact on production. Both technology and management were better mobilised in that year to minimise the adverse effects of drought. Our ability to do so should be improving all the time with the new technology and new management support services which are now being created.

The green revolution thus is not without substance—something which cannot be sustained. It marks the beginning of a modernisation process in our agriculture based on sound policy decisions by the government. Its future momentum would require continued policy support, new technological innovations, specially in the field of soil and water management, and provided these are forthcoming we should be taking it towards its logical path. And that path lies in the closing of the yield gaps. The average crop yields in India, with some exception, are still low when one considers the potential which exists, and this offers us an opportunity to move into the second phase of the green revolution when we should be catching up with the developed countries in the productivity of our agriculture. There is no other alternative. With the kind of population pressures which we would be facing in the next 25 years, we must evolve as advanced an agriculture as in any other country in the world. Fortunately, our resources are considerable. Our soils continue to be some of the most productive in the world even though we have not been very kind to them. The loss of highly fertile topsoils has been accelerated in India in the last 15 years with the rapid disappearance of the forest cover. Our water resources are one of the largest in the world. India should be having more than 110 million hectares of gross irrigated lands by the end of the century with the completion of one of world's most ambitious programmes of development of irrigation. Following the decision of the government to develop these resources and to make massive investments in modern farm input like chemical fertilisers—India today is the fourth largest producer and consumer of fertilizer nitrogen in the world—the country has taken steps to organise a large research infrastructure in order to create a high-yield technology. The ICAR with its network of 40 central institutes and 23 agricultural universities, is one of the world's largest research systems. A fuller exploitation of these resources for continued growth of Indian agriculture and for maintaining the momentum generated in the last 15 years would require one more key input—management support—of which the country is now becoming increasingly aware. The success achieved in the last two years by a more effective mobilisation and distribution of farm inputs has helped to highlight the role of management in our changing agriculture.

2. Farming systems

The policy decisions for the modernisation of Indian agriculture which the country took in the 1960's had implicit in them a major commitment for the restructuring of the developmental administration in the states, making it more effective and responsive in meeting the new needs of farmers and better equipped for the planning and implementation of the new production programmes at the district, block and village levels. The past few years have seen greater conciousness of the need to create new management centres of this kind. However, a basic restructuring of the agricultural extension administration in order to equip it for these tasks has neither taken place, nor the magnitude of the problem clearly understood. The farmers by and large have been able to adopt during the last 15 years only the relatively simple components of the new production technology which were within their reach and could be harnessed largely through their own initiative and efforts. Thus, the farmers have shown a remarkable interest in seeds of the high yielding varieties and in the use of chemical fertilisers. Many of the other components of the new production technology, for example, the plant protection measures, efficient soil and water management and mechanisation of such farm operations as sowing have not been adopted by the farmers on a large scale, although there are some exceptions to this general rule with very impressive results. The exceptions explain the heterogeneity in crop yields in different parts of the country.

The basic reason for the failure of farmers to adopt an integrated approach to crop production is found in the fact that they lacked resources and even more so the agro-services which are a must in a developing country like India for the adoption of several of these components. The new technology may be scale neutral but it is not resource neutral. And more important, it is management intensive. It must receive powerful support not only from agricultural scientists but even more so from modern techniques of management.

The kind of traditional agriculture which most developing countries practise no longer exists in the rest of the world. Agriculture in the developed countries in last 50 years has seen a basic change in its character which we have not fully appreciated. This new agriculture is no longer dominated by farmers in the sense we in India understand. In fact, in countries like the USA, Australia, Canada and in most West European countries, the small farmer with his traditional image, hardly exists. They have been replaced by large farmers who behave more like managers engaged in the organisation of a business. They take the aid of computers in planning their production programmes, in scheduling of their irrigation and in marketing of their produce. They determine their land use patterns, cropping systems and input application based on this kind of analysis. This type of agriculture is best described as an agri-business—a far cry from the subsistence farmers of the developing countries.

The second model of agricultural organisation is presented by the socialist countries of Eastern Europe and the Soviet Union. In these countries, agriculture is organised mostly in the form of large state farms which are run by highly qualified professional managers much in the same way as they run industrial units. The farmers for the most part act as paid workers.

India and other developing countries provide the third model of agriculture as it has evolved over the past several thousand years. Here the land is still in the hands of small farmers who are fiercely conscious of their individual ownership rights. Attempts have been made in many of these countries to bring millions of small and marginal farmers

under some kind of cooperative fold without much success. The best that can be done is the organisation of service cooperatives but even these are not always successful. It is obvious that we have in these countries an imbalance between technology and its management. As long as our farmers were expected to follow traditional practices associated with a low-yield agriculture, they could be expected to provide the limited management support required through their own resources. Today, in order to cope up with the vastly increased human populations in the last 25 years, many developing countries, and this is particularly true of India, are generating a high-yield technology which in many ways is as good as the technology being used by the western farmers. It is clear that the problems of this imbalance can be overcome only by creating a new infrastructure in the form of institutions at the block and the district level, which would offer management support of a more modern kind to millions of these farmers. Above all, this support must take the form of a wide change of agro-services.

The next ten years must see a major strengthening of our blocks so as to make them centers of management of our agriculture both in terms of extension advice and planning of production programmes for millions of farmers and for their implementation through the provision of a wide range of services.

3. Protecting the new genetic potentials

As Indian agriculture begins to close its yield gaps, new problems will arise. Some of these are already beginning to receive attention. First and foremost, there is the problem of price support. The massive procurement operation organised by the Foodgrains Corporation of India points to the magnitude of the problem for which we should be preparing ourselves. The present price support policy in practical terms extends mainly to wheat and rice. It will have to be extended to a wide range of other crops and commodities.

Even as the planners and policy makers grapple with some of these problems, the scientists themselves will be facing new challenges. An important development which could be expected to have serious implications for our future agriculture in India is the replacement during the last 15 years of hundreds of traditional varieties by a handful of high yielding cultivars. Thus, it has been estimated that the Indian farmers may have been growing in their traditional systems of farming as many as 30000 different varieties of rice. Most of these are now being replaced by the new high yielding types so that by the end of the century no more than 500 may be grown, and of these, 20 or so may be saturating large parts of the rice lands in the country.

Introduction of genetic uniformity on this massive scale in our agriculture enhances the possibilities of serious disease and pest epidemics. These epidemics on a minor and major scale have already occurred in India in the last 10 years. Thus, there have been years in the recent past when we may have lost as much as three million tonnes of our wheat crop because of damage caused by leaf and stripe rust diseases and by loose smut.

The short fall in wheat production in Punjab five years ago in relation to the target was largely a function of disease attacking high-yielding varieties like WL 711. Also, the most widely distributed wheat variety in the country, Sonalika, has become increasingly susceptible to disease in the last ten years. The wide spread outbreak of the downy mildew disease of pearl millet provides another example.

The introduction of a new high-yield technology of pearl millet took place in India in the early 1960's when the first hybrid was developed at the Punjab Agricultural University. Subsequently, a number of new hybrids were released—all of them with the same cytoplasmic male sterile parental line as the earlier hybrid. The early 1970's saw a large scale outbreak of disease in the form of downy mildew and ergot which hit all the five hybrids creating a serious problem.

Pearl millet production, which had virtually doubled in the previous years, reaching a record level of nearly seven and a half million tonnes, was reduced to about four million tonnes following the disease outbreak. The last five years have seen a major effort on the part of agriculture scientists in India to diversify the genetic basis of male sterility in this important cereal crop. It has been possible to release a number of new hybrids which are relatively resistant to disease and which are now helping to bring back *bajra* production to the high levels achieved in the early 1970's.

The plant type genes and the broad adaptation characteristic of some of the high-yielding varieties of wheat, rice and other crops developed in recent years have served a most important purpose. While we must recognise this important contribution, it will be a mistake to believe that agriculture in the future could be organised around broadly adapted varieties of this kind. There is a time and place for such varieties and basically they help to borrow time for solutions of a more lasting nature. These solutions must be found in diversifying the genetic base of our crop varieties rather than in narrowing it down, as has happened in the last 15 years. We must evolve a strategy based on erecting genetic barriers against the spread of diseases and pests. The barriers can be provided by genetically diverse varieties which are strategically placed in different parts of the country. I would like to call this approach as the development of a "multi-lineal complex of varieties", each deriving its resistance from a different source and each recommended for a limited area. Thus, in India we have more than 30 wheat varieties recommended for different parts of the country. Varieties of this kind, if they are truly diverse, through their distribution in space and time, provide the best control mechanism against the buildup of a serious disease epidemic. The present wheat varieties as shown in figure 1 may, however, not be very different as most breeders in India have tended to depend heavily on a narrow germplasm base. Recognising this difficulty, the IARI has expanded its wheat improvement programme so as to mobilise genes from as many donor parents as possible. Table 1 lists some of the genes for seedling resistance which the scientists at IARI have found effective against the Indian leaf rust patrogen. It will be noted that some of these genes are effective in the seedling stage against a large number of races. They are obviously of great value for they could provide a source of a more durable type of resistance. The genetic analysis of disease, an example of each is presented above, will have to receive much greater attention in the future. We have been so heavily preoccupied with applied work in the field of crop improvement that we have tended to ignore more basic research in the absence of which progress must slow down. The two schools of research in this field at the IARI and the PAU should be strengthened. We need to involve plant geneticists doing basic work more and more in studies of this kind.

The concept of a multilineal complex of diverse varieties discussed above should be considered as an extension of the multiline varieties concept on a macro scale. A multiline variety simulates in some ways the behaviour of highly heterogeneous land races which farmers have grown for centuries before the start of modern plant breeding programmes. Figure 2 shows how the build up of disease is much less serious in a

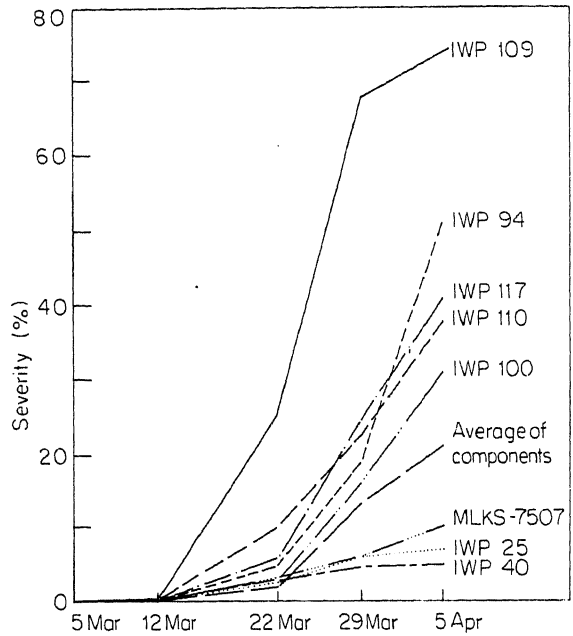


Figure 1. Kalyansona–7507, a multiline version of the famous wheat variety—Kalyansona, shows retarded development of leaf rust compared to the backcrossed component lines which have been combined to form this new variety. Kalyansona–7507 could help to replace kalyansona, which has become highly susceptible to leaf rust (after Rao and Luthra).
Source: Scientific Basis of Indian Agriculture: Seventyfive Years of Service. Indian Agricultural Research Institute, New Delhi, 1980.
Editor: A K Sharma.

multiline variety than in genetically uniform cultivars. A multilineal complex of genetically diverse varieties should help to check the spread of disease from the fields of one group of farmers to those of others in other parts of the country.

4. Seed patents and genetic uniformity

These are the kinds of solutions which scientists would be seeking in a changing world agriculture when the traditional land races and local varieties would be increasingly replaced. Some other developments in recent years, however, raise questions about our ability to do so. More and more, plant breeding in the Western countries is being taken over by private seed companies which insist on patents on the new varieties evolved by them. If the objective is to sell the seeds of a new variety in as large a quantity and in as many different countries as possible to make greater profit through patents as in the case of industrial technology, the emphasis will be on breeding widely adapted varieties. This should reduce the total amount of genetic variability available in a crop.

5. Breeding approaches for greater heterogeneity

An important scientific approach to counter the problem of increasing genetic uniformity of our crops would be to re-examine and re-orient some of our plant

Table 1. Genes for field and seedling resistance found effective against the Indian leaf rust pathogen.

Genes/Stocks	Mean coefficient of infection of four years from five locations*	Races against which effective in seedling stage.
Tc-Lr9	0·55	10, 11, 12, 12A, 17, 20, 63, 77, 77A, 104, 104A, 106, 107, 108, 162, 162A. (All available races in the country)
Tc-Lr14ab–W3531	23·35	10, 11, 12, 17, 20, 63, 104, 106, 108, 162, 162A.
Tc-Lr17	23·70	12, 12A, 12B, 20, 63, 77, 77A–1, 106, 107, 162.
Tc-Lr19	0·05	All available races in the country.
Tc-Lr21	7·65	63.
Tc-Lr22	16·80	None.
Lr24	0·40	**10, 11, 12, 12A, 20, 77, 77A, 104, 104A, 107, 108, 162A.
Lr25	15·65	**12, 12A, 20, 77, 77A, 104, 104A, 107, 162, 162A.
Lr26 'Kavkaz'	0·90	**12, 12A, 20, 77, 77A, 104, 104A, 107, 162, 162A.
Lr27 'Gatsher'	9·9	12, 12A, 20, 77, 104, 104A, 107.
Thatcher (Check)	72·50	—

* Provides a measure of field resistance.
** These include the more virulent and widely prevalent races of leaf rust in India.
(Based on R N Sawhney, IARI, New Delhi).

breeding procedures. Plant breeders all over the world are now becoming conscious of the fact that it is not necessary to evolve varieties which show a high degree of homozygosity and homogeneity. In the case of self-pollinated crops, the recommendation now is that it should be possible to constitute new strains through bulks created in earlier generations rather than proceeding all the way to F8 or F9. The residual variability that such populations maintain is considered a valuable character as long as it does not affect the yield potential or the minimum requirements in terms of crop quality. The legislation on plant breeders' rights would not promote experimental approaches of this kind. A fundamental requirement of a variety to be patented is that it should be quite distinct so that it can be easily identified. Distinctiveness of this kind is possible only when the varieties are taken to extreme levels of homozygosity not only for yield contributing genes but also for most of the other morphological traits which may be of little direct value in the selection of a variety. Thus, plant breeders' rights would not only prolong the breeding programmes, but more important for our present purpose, they would lead to a very high degree of genetic uniformity in the seed population which is ultimately released.

6. Nature of selection pressures and identification of new variability

The high-yield agriculture evolved in the western countries during the last 50 years has derived powerful support from non-renewable resources of energy such as the fossil fuels and the fertiliser feedstocks. This is understandable because energy from these sources was freely available in these years. The result has been that while the modern

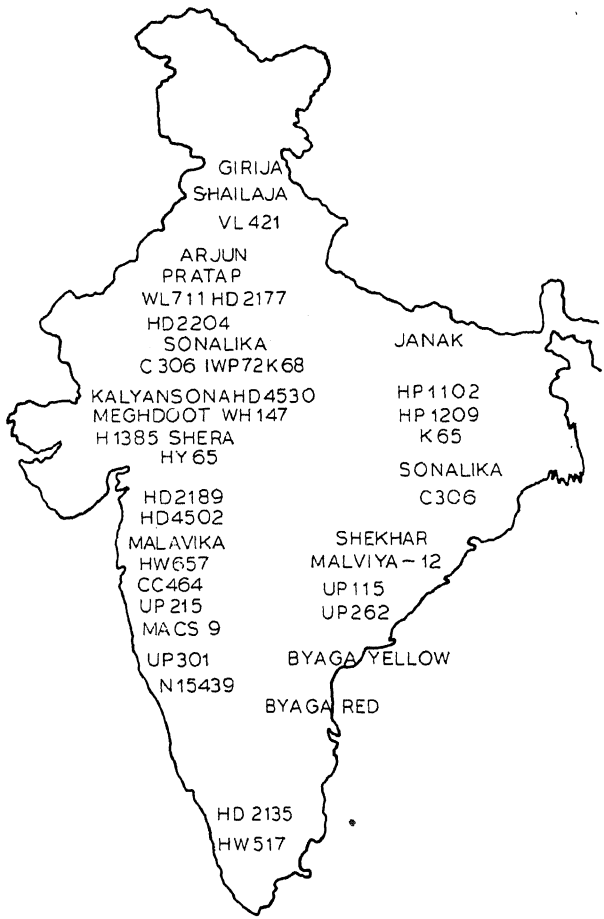


Figure 2. The Indian breeders in the different Agricultural Universities and ICAR Institutes have evolved a large number of high yielding wheat varieties which have been recommended for different parts of the country. These are helping to form a multilineal complex, which needs to be further diversified by mobilising genes for resistance from as many different sources as possible.

agricultural technology is highly productive, it is not necessarily very efficient in terms of the energy input: output ratio. The energy crisis with which the world is faced today and which is likely to worsen in the closing years of the century has focussed attention on the need to evolve a different kind of production technology, which will derive greater support from renewable resources of energy. Operationally this means that plant breeders would be called upon to apply greater selection pressures for attributes like the ability of a genotype to mobolise available nutrients more effectively, to develop complementary interactions with micro-organisms in the rhizosphere for fixation of nitrogen and utilisation of other nutrients, and to show a high degree of resistance to diseases and pests. Theoretically, there is no reason why plant breeders, whether working in the public or the private sector, should not be able to evolve varieties of this kind. In practice, the new approach to plant breeding may not work so well if the objectives are entirely of a short-term commercial nature. It has been pointed

out, for example, that a private seed company may be interested not so much in the efficiency of the production process as in the productivity itself, irrespective of the demand on non-renewable inputs. If this happens, a great deal of genetic variability which could be potentially of very great value to world agriculture in the years to come will remain unidentified. It may even be eroded because of genetic drift simply because the present selection pressures do not favour it. The net effect would be a narrowing down of the genetic resources of the kind which plant breeders would need most if a new direction is be given to crop improvement programmes of the future.

7. India's plant genetic resources

Our changing agriculture with major emphasis on development and release of high-yielding varieties of crop plants also raises the more fundamental question regarding conservation of our genetic resources. These resources are basically a product of evolution over millions of years. The first group of farmers and their descendants in all parts of the world including the valleys of Euhrites and Tigris, where the process of domestication of crop plants first started, must be given the credit for recognising the value of genetic resources, and for selecting the most useful of them for the purpose of agricultural production. The successive generations of farmers have continued to select but not with the kind of intense selection pressures which we are now beginning to apply. Their efforts have resulted in the evolution of hundreds of land races. Are we now to destroy all this useful reservoir of genetic material for the sake of our immediate gains? It is well known that while these primitive varieties may not be high yielding and cannot respond to our present needs of agricultural production, they do contain some very useful genes, which we cannot afford to lose. Since traditional agriculture has been practised without investment in modern farm inputs like chemical fertilizers, pesticides and irrigation, the earlier generations of farmers had to select for vegetative vigour, hardiness and for tolerance to drought, pests, pathogene and weeds. In this way, a wide array of valuable genes has been built up in our crop plants over the centuries. It is obvious that these must be preserved for our future needs. Even as we embark on a high-yielding varieties programme, the need for these genes is already been felt. A striking example of this is provided by the outbreak of the brown plant hopper in recent years—a pest which has attacked the new varieties evolved by the International Rice Research Institute. The outbreak has threatened the agriculture of several countries in South–East Asia and South Asia where large areas are planted with some of these varieties. The community of rice breeders has mounted one of the largest programmes of resistance breeding against this pest. Interestingly, the sources of resistance have been located in some of the primitive varieties, particularly those of the Patambi group in Kerala in South India. The problem, of course, is not unique to India and there has been a worldwide concern about the loss of genetic resources. The creation of The International Board of Plant Genetic Resources in FAO by the Consultative Group on International Agricultural Research has been the first major step in preventing this loss. The Board in the last 10 years has arranged and helped to finance some 250 collecting missions in more than 70 countries. It coordinates plant genetic resources activities on a global basis. Scientists in almost 100 countries collaborate with the Board for the purpose of collecting, conserving, storage, documentation and evaluation of these resources. In India, the focal point of such

activities is the newly established Bureau of Plant Genetic Resources under the ICAR. It is clear, however, that the Bureau by itself cannot do much unless it collaborates with scientists in the different Agricultural Universities and other ICAR Institutes in the country. India must organise a nationally coordinated research project on plant genetic resources if this precious heritage is to be saved for posterity.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 475–485.
© Printed in India.

Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue

WILHELM NULTSCH and MARIA HÄDER

Fachbereich Biologie, Lehrstuhl für Botanik der Philipps-Universität Marburg, Karl v. Frisch-Str., 3550 Marburg 1, Federal Republic of Germany

Abstract. The colourless, stigma-free flagellate *Polytomella magna* which does not show natural responses to light becomes sensitive to light in the presence of photodynamically active dyes, such as riboflavin, methylene blue, toluylene blue, thionine, toluidine blue, neutral red and rose bengal, provided that oxygen is present. This reaction resulting in a light avoiding response depends neither on the absorption characteristics nor on the redox properties of the dyes. Detailed studies with methylene blue have shown that this response depends on the photon fluence rate and the dye concentration as well. The action spectrum of the photodispersal calculated on the basis of photon fluence rate-response curves is essentially identical with the absorption spectrum of methylene blue, *i.e.* no responses occurred below 530 and above 700 nm. Pre-irradiation experiments have shown that methylene blue is photobleached and, hence, inactivated in the presence but not in the absence of oxygen. Moreover, it has been demonstrated that the methylene blue mediated responses are chemotactic rather than photophobic ones. These experiments confirm the results of earlier investigations with riboflavin.

Keywords. *Polytomella magna*; flagellates; methylene blue; photodispersal; photodynamic action; chemotaxis.

1. Introduction

Photodynamic effects, resulting in photokilling, were first described by Raab (1900). Its oxygen dependence was observed by Jodlbauer and Tappeiner (1905). The term "photodynamic action" was introduced by Tappeiner and Jodlbauer (1905). Photodynamically induced movement reactions of microorganisms such as the bacterium *Spirillum volutans*, the ciliate *Paramecium caudatum* and the diatom *Navicula* sp. were studied extensively by Metzner (1920, 1921, 1924). Though he discussed the possibility that these reactions could be chemotactic responses caused by dye peroxides, he concluded that the dyes were taken up by the cells and incorporated into either the cytoplasmic membrane or the cytoplasm, acting as artificial photoreceptors and triggering a photophobic response. He called this phenomenon "induced phototaxis".

Though the photokilling of several different organisms in the presence of photodynamic dyes was studied frequently during the last decennia (*c.f.* Ito 1977; Spikes 1982 and references therein), photodynamically induced behavioural responses of microorganisms have not been investigated until recently. The first recent paper was published by Nultsch and Häder (1984) who investigated the effect of riboflavin as a photosensitizer on the movement of the colourless flagellate *Polytomella magna*. They have shown that the photodispersal is not the result of a photophobic response but is caused by negative chemotaxis to a photoproduct formed in the presence of the dye at high fluence rates. The following experimental results support this conclusion:

(i) Riboflavin is not taken up by the cells in spectrophotometrically detectable amounts. (ii) The responses disappear immediately after the dye has been removed. (iii) Some time after the beginning of the experiments the cells respond already in the dark surrounding field. (iv) The dispersal from the irradiated field continues to occur for about a min or longer after the active white light has been switched off. (v) Pre-irradiated riboflavin filled in a capillary tube, causes negative chemotaxis, if observed with inactive red light.

Moreover, these authors gave some experimental evidence that this chemotactically effective photoproduct is hydrogen peroxide (H_2O_2). Similar results have been obtained by Häder (1982, 1984) who showed that the behavioural responses of the colourless flagellate *Peridiniopsis berolinensis* photodynamically induced by riboflavin, *i.e.* photoaccumulation at low but photodispersal at high photon fluence rates, are also caused by positive and negative chemotaxis to a photoproduct.

More recently, Nultsch and Kumar (1984) have shown that the photoproduct, apparently H_2O_2, is produced in a so-called type I (free radical) reaction (*c.f.* Ito 1983 and references therein), while the type II (singlet molecular oxygen mechanism) could be excluded. Moreover, both singlet oxygen and superoxide could be also excluded as possible active photoproducts. These results have been supported further by Kumar and Nultsch (1985).

The aim of this study was to find out whether other photodynamic dyes, especially methylene blue, show the same effects as riboflavin, and whether the results obtained with riboflavin can be generalized as a concept to explain the mechanism of photodynamically induced behavioural responses of microorganisms.

2. Materials and methods

2.1 *Culture*

As in earlier experiments the colourless and stigmaless strain L 63–4 of the flagellate *Polytomella magna* Pringsheim (Culture Collection Göttingen, FRG) was grown in an organic medium (Nultsch and Häder 1984) in 250 cm^3 Erlenmeyer flasks, each containing 100 cm^3 nutrient medium. Samples were taken at the end of the logarithmic phase, *i.e.* after 2 days of growth. The pH increased during this time from 6·8 to 8·4–8·6. The cell density was about 2×10^6 cells/cm^3. For further details see Nultsch and Häder (1984).

2.2 *Chemicals*

Riboflavin, methylene blue, toluylene blue, thionine, toluidine blue Hoyer, neutral red (Merck, Darmstadt, FRG); lumichrome, lumiflavin (Sigma, München, FRG); rose bengal (Fluka, Neu-Ulm, FRG).

2.3 *Experimental*

The dyes were dissolved in culture medium and mixed with the culture to the desired final concentration. The pH was adjusted to 8·5 with 0·038 M Tris buffer. 0·1 cm^3 of this preparation containing 2×10^5 cells was transferred on a microscope slide, covered by a cover slip and sealed with vaseline. Photobehaviour was investigated

either with the light field method or by studying the movement behaviour of single cells at a light-dark boundary. In the light field method a white light field (fluence rate about 950 Wm^{-2}) was projected onto the cell preparation, and the movement of the cells was recorded with a microvideo system (figure 1). The number of cells seen in the light field was counted by means of single frame analysis at intervals of 5 sec. Monochromatic light was produced by inserting Schott (Mainz, FRG) interference filters (half band width about 10 nm). In the light-dark boundary studies one half of the light field was covered with a far red cut off filter (715 nm, Schott, Mainz, FRG). Far red is photodynamically ineffective but allows video recording. For further experimental details see Nultsch and Häder (1984).

3. Results

3.1 *White light experiments*

The behavioural responses of the *Polytomella* cells induced by the photodynamically active dyes (riboflavin, methylene blue, toluylene blue, thionine, toluidine blue, neutral red, rose bengal) resulted always in a dispersal from the light field, as shown in figures 2 A–C, so that at the end of the experiment the light field was empty. If at this time the diaphragm of the microscope was opened, a broad empty area surrounding the light field could be observed (figure 2 D). This indicates that the behavioural responses of *Polytomella* are caused by diffusible photoproducts of the dyes acting as chemotactic repellents. This confirms the earlier observations by Nultsch and Häder (1984), who concluded that these responses are of chemotactic nature. Moreover, the swimming behaviour indicates a chemotactic rather than a chemophobic response.

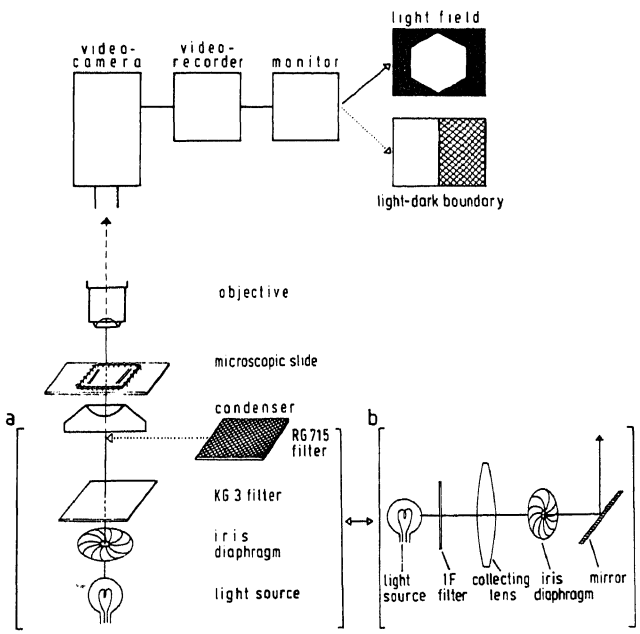


Figure 1. Schematic diagram of the microvideosystem. **a.** Set-up for white light experiments. **b.** Set-up for monochromatic light. (after Nultsch and Häder 1984).

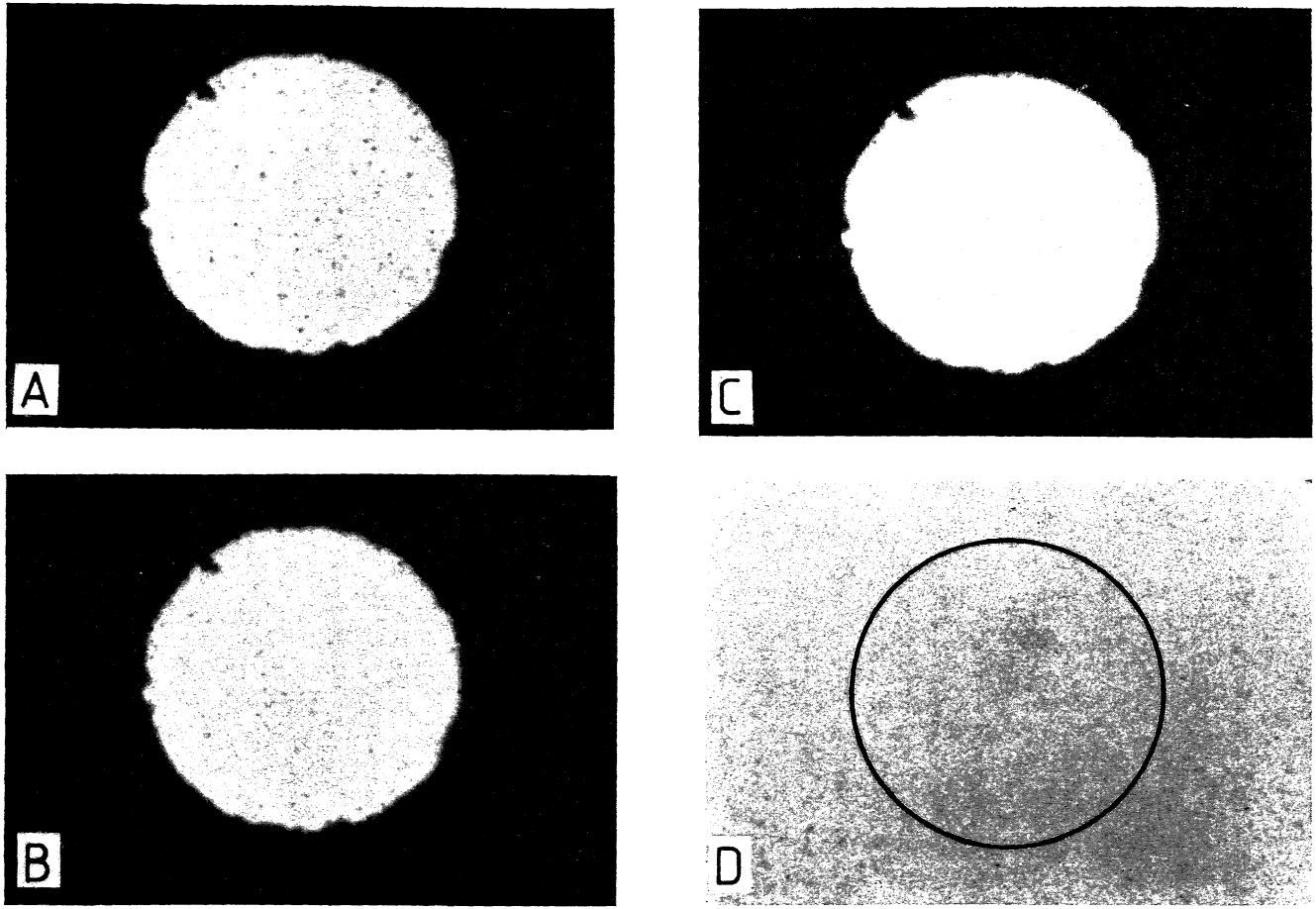


Figure 2. Distribution of *Polytomella* cells in the light field. **A.** At the beginning of the experiment (RG 715 nm filter inserted, no reaction). **B.** 12 sec, **C.** 22 sec after RG 715 nm was removed. **D.** Diaphragm opened after 25 sec, showing the empty zone around the light field, indicated by a black circle.

As shown in figure 3, different types of dyes are active and the absorption maxima vary between 440 and 665 nm, the redox potentials between −0·2 and +0·1 V, and even dyes without redox properties such as rose bengal cause photodispersal. Consequently, neither the wavelengths nor the redox properties of the dyes can be responsible for the photodynamic effects. In this connection it must be mentioned that these behavioural responses induced by light and dyes depend on the presence of oxygen, as already observed by Metzner (1920).

As one would expect, the production of the chemotactic agent and hence, the photodynamically induced response depends on the irradiance. In 10^{-4} M methylene blue (MB) solution the threshold value of the photodynamically induced response is about 3000 lx (figure 4). With increasing irradiance the responses become more pronounced, reaching a saturation level of about 100 % at 70000 lx. As seen in the same figure, the addition of Tris does not influence the reactivity. In the absence of MB no response can be observed up to about 500000 lx.

The kinetics of the dispersal from a light field caused by MB are shown in figure 5. Whereas at 190 Wm^{-2} the curve is steep, reaching complete dispersal (cell number zero) 40 sec after the onset of white light illumination, the slope of the curves measured

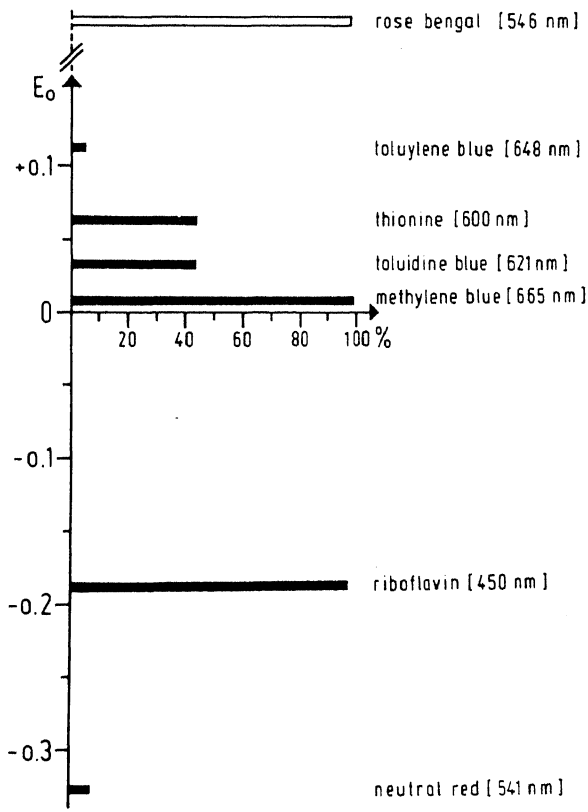


Figure 3. Photodynamic effectiveness resulting in dispersal reactions of *Polytomella* from the white light field (950 Wm^{-2}) in percent (abscissa) versus normal potential (E_0) of the tested dyes (ordinate). Absorption maxima of the dyes in square brackets.

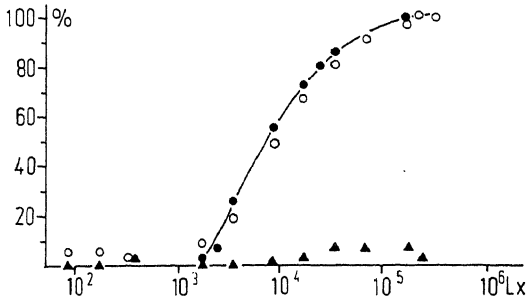


Figure. 4. Irradiance dependence of the photodynamically induced behavioural responses, measured at a white light-dark boundary in the presence of 10^{-4} M MB without Tris (o) and with Tris (•). Control without MB and Tris (filled triangles). Abscissa: irradiance in lx; ordinate: percentage of reacting organisms.

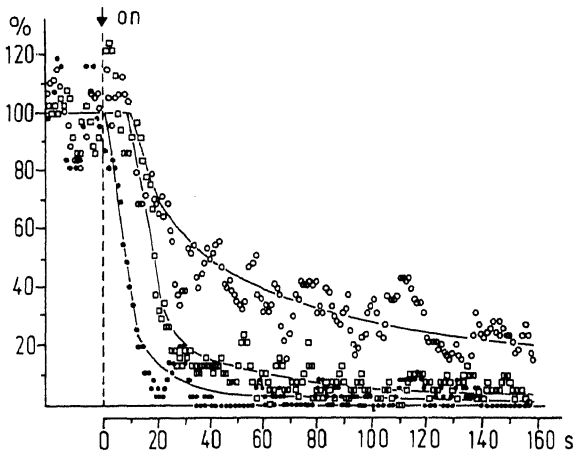


Figure 5. Kinetics of the dispersal from a light field caused by 10^{-4} M MB at different photon fluence rates: (•) 190, (□) 95, (o) 46 Wm^{-2}. Abscissa: time in sec; ordinate: number of cells in the light field in percent of the control (RG 715 cut-off filter). Arrow: white light on.

at lower fluence rates is not as steep, so that complete dispersal is reached after about 120 sec at 95 Wm^{-2}. At 46 Wm^{-2} about 20 % of the cells were still in the light field after 180 sec.

The photodynamic effect depends also on the dye concentration. For MB the minimal concentration causing significant reactions is 10^{-6} M, while the maximum (100 % reaction) is reached at about 10^{-5} M (figure 6).

3.2 *Action spectrum*

In order to obtain an action spectrum of the MB induced photodispersal photon fluence rate-response curves were measured at different wavelengths. Some of them are shown in figure 7. The reciprocal values of the fluence rates causing 50 % reaction were plotted

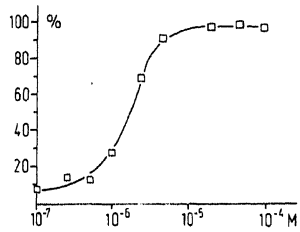


Figure 6. Dependence of the photodynamically induced behavioural responses on the MB concentration, measured at a white light (950 Wm^{-2}) -dark boundary in the absence of Tris. Abscissa: MB concentration in M; ordinate: percentage of reacting organisms.

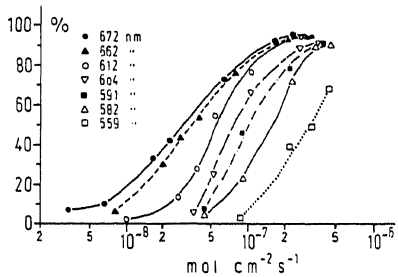


Figure 7. Photon fluence rate-response curves of the photodispersal of *Polytomella* at several different wavelengths in the presence of 10^{-4} M MB. Abscissa: photon fluence rate in mol cm^{-2} sec^{-1}; ordinate: percentage of reacting organisms.

versus wavelength. The resulting action spectrum is shown in figure 8. As can be seen, it is essentially identical with the MB absorption spectrum.

3.3 *Pre-illumination experiments*

Since the photodynamic effect disappears after a longer irradiation (several hours) even in the presence of oxygen, experiments with MB solutions which were pre-illuminated in the absence of *Polytomella* cells were carried through. 10^{-4} MB was pre-illuminated for 48 hr with 950 Wm^{-2} white light either in a closed, anaerobic cuvette or in an open cuvette in presence of oxygen. These solutions were used for light field experiments as usual, and the results were compared with those obtained with fresh MB solutions. The results (figure 9) show that MB pre-illuminated anaerobically still has a strong photodynamic effect, *i.e.* complete photodispersal is reached 60 sec after the onset of white light illumination. On the contrary, in the aerobically pre-illuminated solution only a slight photodynamic effect can be observed after 150 sec. A mixture of fresh and aerobically pre-illuminated MB solutions (1:1) has the same effect as fresh MB, indicating that the pre-illuminated MB does not make the cells insensitive.

The ineffectiveness of the aerobically pre-illuminated MB is apparently due to the photodestruction of the dye, indicated by complete photobleaching (figure 10). As can

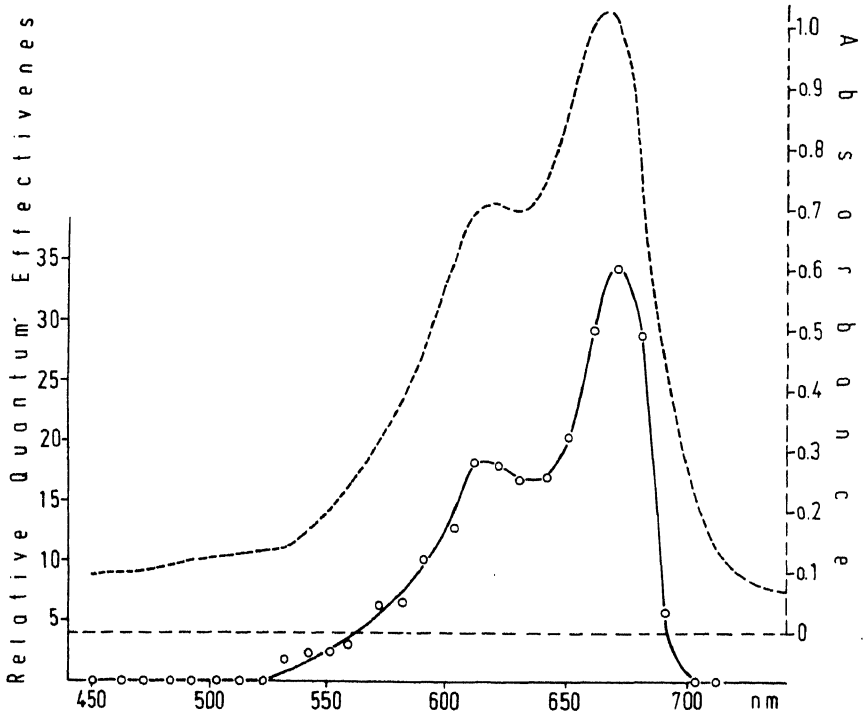


Figure 8. Action spectrum of photodispersal calculated from photon fluence rate-response curves (o), (—) and absorption spectrum of MB (---). Abscissa: wavelength in nm; ordinate: relative quantum effectiveness and absorbance, respectively.

be seen in the same figure, MB pre-illuminated under anaerobic conditions is photobleached only in part.

3.4 *Experimental evidence for chemotaxis*

Immediately after the onset of white light illumination, i.e. after the removal of the RG 715 filter, the cells suspended in 10^{-4} M MB responded inside the light field. With increasing irradiation time the zone of response was shifted to the border of the light field, and finally the cells responded outside the light field, so that the light field was surrounded by a cell free area, as shown in figure 2 for riboflavin. This behaviour is indicative for a chemotactic reaction to an agent which is produced inside the light field in the presence of the dye, but then spreads by diffusion.

If the kinetics of the photodispersal (950 Wm^{-2} after removal of RG 715) and of the refilling (RG 715 inserted) of the light field in the presence of 10^{-4} M MB are compared (figure 11), it is seen that the time for the complete photodispersal was 10 sec, whereas after reinserting the RG 715 filter the light field remained empty for further 10 sec. Then the cell number in the red light field increased gradually, and the control value was reached again after about 90 sec, *i.e.* the kinetics of photodispersal and refilling differ by a factor of 10. In the case of photophobic responses the refilling time of light traps is of the same order of magnitude as the one of photodispersal. Thus, the results

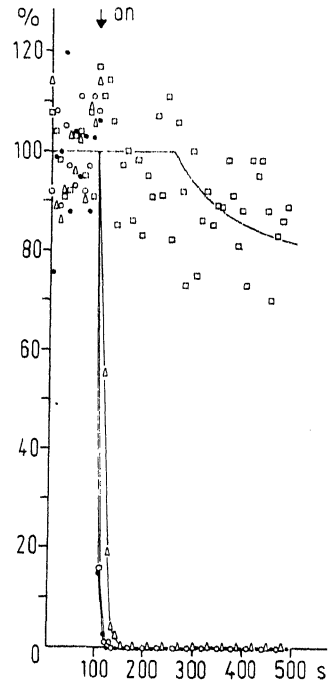


Figure 9. Kinetics of the dispersal from a light field (950 Wm^{-2}) in fresh and pre-illuminated (48 hr) 10^{-4} M Tris buffered MB solutions. Fresh MB (dots), anaerobically pre-illuminated (triangles), aerobically pre-illuminated (squares), mixture (1:1) of fresh and aerobically pre-illuminated (circles). Abscissa: time in sec; ordinate: number of cells in the light field in percent of the control (RG 715 cut-off filter).

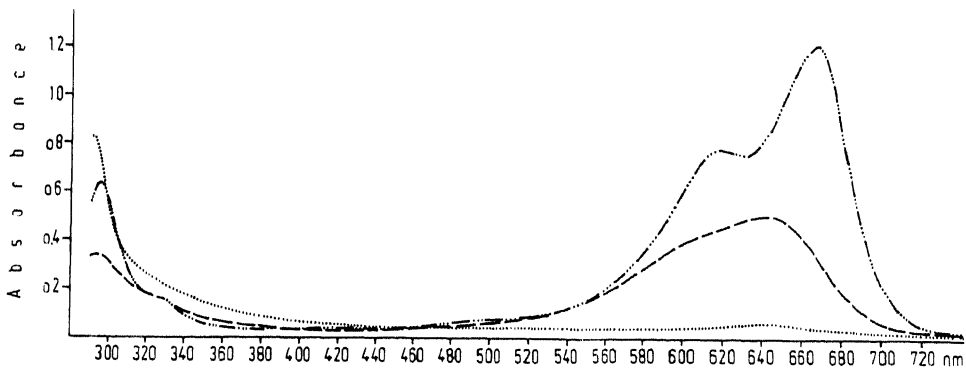


Figure 10. Absorption spectra of $2{\cdot}5 \cdot 10^{-5}$ M MB in solutions in Tris buffered nutrient medium. Fresh (— . . . —), aerobically pre-illuminated (. . .), anaerobically pre-illuminated (– – –). Abscissa: wavelength in nm; ordinate: absorbance.

of this experiment are also strongly indicative of a chemotactic response to a photoproduct.

Finally, if MB is pre-irradiated with white light (950 Wm^{-2}) for 18 hr and filled into a capillary tube, which is inserted in the preparation of organisms on a microscopic slide, negative chemotaxis can be observed.

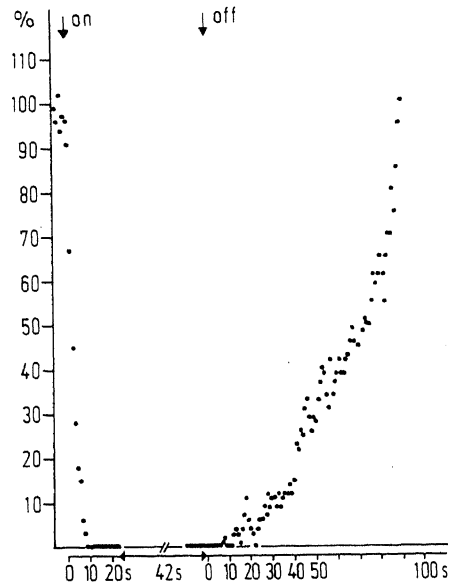


Figure 11. Comparison of the kinetics of photodispersal after onset of white light illumination (950 Wm^{-2}, RG 715 filter removed) and refilling of the light field after switching off the white light by re-inserting RG 715 filter, in the presence of 10^{-4} M MB. Abscissa: time in sec; ordinate: cell number in the light field in percent of the control (RG 715 cut-off filter).

4. Conclusions

The experiments reported in this paper have shown that methylene blue causes the same photodynamic effects as riboflavin. The irradiance response, the dye concentration dependence and the kinetics of photodispersal are similar, though MB seems to cause even stronger photodynamic effects than riboflavin. As in riboflavin, the action spectrum indicates that monochromatic light is active according to its absorption by the dye. Photodestruction of the dye destroys its activity.

Moreover, the chemotactic character of the behavioural responses has been demonstrated also for MB:

(i) The cells respond inside the light field at the beginning, but outside the light field some time after the beginning of the experiments. This excludes a photophobic response which occurs immediately at the light-dark border.

(ii) The kinetics of the photodispersal differ from those of the refilling of the light field, after the active light has been switched off, by about one order of magnitude, whereas the dispersal and refilling times are about equal in photophobic experiments.

(iii) A capillary tube filled with pre-irradiated MB causes negative chemotaxis.

Thus, we may conclude that the behavioural responses of *Polytomella magna* caused by both riboflavin and methylene blue are chemotactic responses to a photodynamically formed substance which is apparently H_2O_2, produced in a type I reaction. It seems probable that this mechanism is common to all dyes causing photodynamic behavioural responses.

Acknowledgements

The authors are indebted to the Deutsche Forschungsgemeinschaft for financial support and to the Institut für den wissenschaftlichen Film, Göttingen (FRG) for the photographs taken.

References

Häder M 1982 *Lichtinduzierte Bewegungsreaktionen farbloser Flagellaten nach Zugabe photodynamisch wirksamer Farbstoffe* PhD Thesis Marburg/Lahn

Häder M 1984 Light-induced chemotactic reactions in the dinoflagellate, *Peridiniopsis berolinensis*; *Photochem. Photobiol.* **40** 533–537

Ito T 1977 Cellular and subcellular mechanisms of photodynamic action: the $^1O_2$ hypothesis as a driving force in recent research; *Photochem. Photobiol.* **28** 493–508

Ito T 1983 Photodynamic agents as tools for cell biology; in *Photochemical and photobiological reviews*. (ed.) K S Smith (New York: Plenum) Vol. 7 pp. 1411–1486

Jodlbauer A and Tappeiner A von 1905 Die Beteiligung des Sauerstoffs bei der Wirkung fluorescierender Stoffe; *Dtsch. Arch. Klin. Med.* **82** 520–546

Kumar H D and Nultsch W 1985 Effects of saffron extract and carotenoid preparations on the photodynamically induced chemotactic response of *Polytomella magna*; *Photobiochem. Photobiophys.* (in press)

Metzner P 1920 Über die Wirkung photodynamischer Stoffe auf *Spirillum volutans* und die Beziehungen der photodynamischen Erscheinung zur Phototaxis. I. Mitt.; *Biochem. Z.* **101** 33–53

Metzner P 1921 Zur Kenntnis der photodynamischen Erscheinung: Die induzierte Phototaxis bei *Paramaecium cuadatum*. II. Mitt.; *Biochem. Z.* **113** 145–175

Metzner P 1924 Zur Kenntnis der photodynamischen Erscheinung. III. Mitt.: Über die Bindung der wirksamen Farbstoffe in der Zelle; *Biochem. Z.* **148** 498–523

Nultsch W and Häder M 1984 Light-induced chemotactic responses of the colorless flagellate, *Polytomella magna*, in the presence of photodynamic dyes; *Arch. Microbiol.* **139** 21–27

Nultsch W and Kumar H D 1984 Effects of quenching agents on the photodynamically-induced chemotactic response of the colorless flagellate *Polytomella magna*; *Photochem. Photobiol.* **40** 539–543

Raab D 1900 Über die Wirkung fluorescierender Stoffe auf Infusorien; *Z. Biol.* **39** 524–546

Spikes J D 1982 Photodynamic reactions in photomedicine: in *The science of photomedicine*, (eds) J D Regan and J A Parrish (New York: Plenum Press), pp. 113–144

Tappeiner H von and Jodlbauer A 1904 Über die Wirkung der photodynamischen (fluorescierender) Stoffe auf Protozoen und Enzyme; *Dtsch. Arch. Klin. Med.* **80** 427–487

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 487–504.
© Printed in India.

Additional DNA sequences—Topography, property, role and evolutionary significance

ARUN KUMAR SHARMA

Centre of Advanced Study in Cell and Chromosome Research, Department of Botany, University of Calcutta, 35, Ballygunj Circular Road, Calcutta 700019, India

Abstract. A high percentage of DNA sequence noted in higher organisms is highly repetitive in nature. Such sequences may be highly homogeneous, moderate, minor and inverted. Those sequences are found in different loci including introns, spacers, transposons, in addition to their interspersion with unique sequences. Such sequences share the property of amplification, dispersion and possibly mobility. The term 'Dynamic DNA' has been proposed to account for their varied property. Their presence may account for "C Value Paradox" in eukaryotes. Evolutionary advance is also associated with increase or decrease of repeated sequences.

Keywords. Additional DNA sequences; chromosome structure; transposons; spacer; genetic elements.

1. Introduction

The study of chromosome architecture has acquired special dimensions following the tremendous ramification of physical and chemical methods in the analysis of the functional segments. Even in the eukaryotic systems, where the structure is truly a chromosome, not merely a genophore, the range of diversity is wide, with organisms like *Spirogyra* and yeast at one end of the spectrum and primates at the other. Inspite of progressive complexity and diversity, however, their elegance and fidelity in adhering to the common genetic principles, are indeed remarkable. In these systems, the chromosomes are visualized as giant complex molecules of DNA, composed of less complex genes, of variable length. Refinements in ultra-structural techniques (Sharma and Sharma 1980) have led to the resolution of chromosomes into fibrils 20–30 Å in diameter, folded several times to give the ultimate thickness of 100 Å. Cytologically, it is a continuous deoxyribonucleoprotein fibre having alternating condensed and uncondensed segments. Pulse labelling, as well as high resolution autoradiography, has revealed that chromosomes are made up of several replicons which undergo simultaneous replication during reproduction. The application of X-ray diffraction analysis has shown the chromosome to be composed of a bead-like structure, each bead being designated as a *nucleosome*. Five histone fractions are found in the nucleosomes. Four of these namely, H-2a, H-2b, H-3 and H-4 form an octamer, H-1 being associated as a linker. The DNA segment of approximately 200 base pairs is coiled round the nucleosomes. A large number of these base pairs are highly conserved, the rest being variable and associated with the linker. The DNA wrapped around the octamer of histones makes the diameter of the spherical structure to be 100 Å, thus agreeing with the earlier demonstration of the same diameter of the DNA fibre. Thus the proteins entering into the chromosome architecture of the nucleosomes are also repeated units, whose composition may be variable in certain cases.

2. Complexity of chromosome structure

The complexity of chromosome structure at the eukaryotic level has been demonstrated during the last decade. A series of observations indicates the presence of intervening sequences or *introns*, implying that the eukaryotic genes are not continuous but rather discontinuous, the essential components being termed as *exons*. In other words, the gene may be a split one, with introns and exons alternating with each other. The discovery of split genes came from the observation that the primary transcript is much longer than the translatable transcript and that a significant amount of processing may take place between transcription and translation.

The other discovery which has aided interpretation of chromosome structure is the identification of multiple copies of similar DNA sequences in the chromosomes of higher organisms. These sequences are to some extent analogous to the polygenes. However, the small, similar and supplementary effects characterising the polygenes are not necessarily comparable to DNA repeats, which may be intragenic as well. Such repeated sequences may be very homogeneous, as initially found in *Xenopus* and later in other organisms and may be termed *satellite* DNA. They may be made up of lesser number of copies for which the term *moderate* or *minor* repeat is applied. Repeated segments may be inverted or *palindromic* in nature. The modern concept or chromosome structure, as demonstrated by different groups of workers, including our own, is that the chromosome represents a linear array of independent replicons with major repeats at a specific locus and minor or moderate repeats interspersed. The palindromes occur at ends or intercalary positions at specific loci.

The discovery of repeated sequences has considerably assisted the interpretation of chromosome structure in terms of function and evolution. The enormity of the problem can be judged from the very fact that in the human system, regarded as highly advanced in the hierarchy of evolution, nearly 40 % of the DNA is repetitive in nature. In several plant species, 70–75 % of DNA are repetitive, and in *Drosophila* such sequences constitute almost 50 % of the genome. In some mammalian systems, nearly 95 % of the total DNA is repetitive. In the human system, a cell contains almost 7·1 pico-gram of DNA with $7{\cdot}1 \times 10^9$ base pairs. Theoretically a human cell can accommodate a few million genes based on a calculation of approximately 1200 base pairs for a gene. If only the unique sequences are expressed, which form a fraction of the total genome, approximately 60000 essential genes can be estimated; the rest of the DNA is in the repetitive fraction. It is easy to understand the rationale behind the low number of essential genes. Had all the several million genes leading to genetic overload (Kimura 1968) been functional, it would have led to chaos and lethality according to known mutation rates of the genes.

Additional elements in chromosomes include the repeated sequences and amplified copies. The latter represent *lengthwise*, as against *tandem* duplication of DNA segments in the former. Both may however, be involved, as in r DNA locus in Drosophila (Kunz *et al* 1982). Irrespective of the fact that repeated sequences are of nearly universal occurrence, their function remains a mystery. Various authors have attributed different functions to the repeated sequences and several epithets have also been applied. However, if the concept of additional genetic elements in the chromosomes is accepted then both phylogenetic and ontogenetic increases are to be taken into account.

3. Ontogenetic increase

The importance of additional DNA during development and differentiation has been recognised (Nagl 1972). Endomitotic replication of chromonema has been recorded during differentiation. This has been attributed to the need for additional protein synthesis whereas some consider this method of replication as a mechanism for the supply of fresh strands for continued transcription when the original strand is no longer operative (Evans and Van't Hof 1975; Sharma 1976; 1978a, b). The entire process is under genetic control and the need for such replication is entirely dependent on the genetic constitution of the species which determines the period during which the genes may continue to transcribe without undergoing replication. Several cases have been recorded from this laboratory where such amplification of DNA has been shown to be associated with differentiation (Banerjee and Sharma 1979; Sharma and Mookerjea 1954). The amplified sequences need not necessarily involve the entire genome and therefore variable contents of DNA may characterise the different organs of individuals (Banerjee and Sharma 1979; Sau *et al* 1980; Sharma 1983).

Nagl (1972) suggested that plants normally possess a mechanism for phylogenetic increase in DNA as exemplified by the presence of polyploidy, generative polyploidy and repeated sequences. However, increase in DNA may involve multiple copies of the same or introduction of new information contents. Polyploidy on the other hand merely involves addition of back up copies. Phylogenetic increase does not affect constancy of the amount of DNA in different cells. In addition to earlier works (see Sharma 1978a, b), in later years ontogenetic increase of DNA has been reported in several organisms. Sekerka (1981) reported a change in DNA content of interkinetic nuclei of the epicotyl of *Vicia faba* during dedifferentiation. Such amplification during root development of *Allium cepa* has also been noted (Cremonini *et al* 1981). In *Arachis hypogea* a variation from 2C to 8C DNA was recorded in various tissues, the minimum being in root and shoot apices and leaf tissues (vide Sharma 1978a, b).

Such high DNA values have been correlated with protein synthesizing capacity. In *Drosophila* the amplification of two chromosomal domains containing chorion genes has been observed. These genes undergo amplification in ovarian follicles prior to expression in late oogenesis. In the scutellum of *Triticum durum*, a difference in DNA content has been recorded, along with different histone-DNA ratios, in different tissues following ageing. Such repeats may originate out of tandem duplication and unequal crossing over. Species, in which the mechanism of phylogenetic increase is not operative, resort to amplification of DNA during ontogeny. This issue however, needs to be thoroughly investigated.

The significance of high frequency repeats has not yet been fully established. The increase in copies of existing sequences may form the basis for the generally accepted existence of families of repeated sequences, provided however, such multiple copies remain long enough to become a family of repeated sequences. It would imply that *Xenopus* ribosomal genes may form a family. However, evidences for such a mechanism are yet to be obtained.

Several functions have been attributed to these genes including those of regulation, maintenance of chromosome structure, control of crossing over and differentiation of chromosome segments. Such repeats have also been claimed to play an important role in serving as reservoir of loci for the accumulation of mutation or even as padding to keep the chromatin in the folded state.

4. C-value paradox

The repeated sequences have given rise to a paradoxical situation with regard to the genomic content of different species. In most eukaryotic systems, the amount of DNA in the genome, otherwise known as *C-value*, is much greater than the total number of structural genes as already mentioned in case of human cells. This has led to the situation termed as *C-value paradox*. It is indeed very difficult to correlate the degree of differentiation with the amount of DNA in an organism. The best examples are the lilies and amphibians where the range in amount of DNA is 20–100 pico-gram as compared to 2–3 pg in mammals and 7 pg in human system (Lewin 1980). Evidently the repeated sequences are involved with genes which do not code for structural proteins but which lead to variability of DNA values in different hierarchy of organisation. This redundancy is suggested in view of the absence of information content. The C-value paradox may not hold good to the same extent for all members of a species. The extent of variability in C-values at the intraspecific level, so far noted in certain organisms, is not fully known (Kurnitt 1979; Patton and Sherwood 1982; Sherwood and Patton 1982). Even in the human system, quantitative variations of the genome have been recorded (Bostock *et al* 1978; Gosden *et al* 1981). A correct interpretation of the significance of C-value will have to await an analysis of its variability in natural populations at the intraspecific level.

4.1 *Satellite DNA*

Satellite DNA (Sat-DNA), with high homogenous repeats, has been located in several organisms. In pea, four fractions of r RNA were observed to hybridise with the main band (Wall and Bryant 1981). In *Drosophila viridis* sat-DNA s are very specific in location and are rich in A-T sequences. They are consistently located on the sides of the nuclei nearest to the vitelline membrane. Highly repetitive satellite DNA sequences have been found in cucurbitaceous species. The restriction pattern suggests an internal Hind III sequence of repeats with 380 base pairs in *Cucumis melo*. In *Cucumis sativus*, on the other hand, excision results into repeated units of 180 base pairs. A certain amount of homology between the satellites of the two species has been recorded, together with the properties of amplification and dispersion. Satellite DNA s have also been traced through *in situ* hybridization in human acrocentrics, specially on the short arm. Some of them exhibit some heterogeneity in their DNA content. In this connection, the intensity of fluorescence has not been considered as a proper criterion for detecting homogeneity of satellites (Gosden *et al* 1981).

Highly repeated sequences have been recorded in the human Y chromosome and related sequences in higher primates (Cooke *et al* 1982). The human Y has been shown to contain 2000 copies of tandem repeats, 2·47 kilo base long, constituting about 20% of the DNA of the chromosomes. Sequences are also distributed in tandem arrays in a number of autosomes, through amplification and dispersion in gorilla and chimpanzee. Repeats, though conserved, have undergone a continued process of dispersion during evolution. In man, the block homogeneity in Y has been maintained through unequal crossing-over. The increase in the number of copies of a short repeated sequence may in fact be a common event in evolution, ultimately increasing the sequences to the extent that they may be detected as satellite DNA sequences. A satellite is historically a CsCl-density satellite. In case there is no density difference, a satellite is not observed on

CsCl, as in green monkey, but a reassociation or restriction enzyme is given for a family of repeats, whose members are not exact copies of each other.

4.2 *Other reports*

In addition to highly homogenous ones, *moderate* and *minor* repeated sequences form a significant part of repeated DNA in biological systems (Davidson *et al* 1983). In general, moderate and minor repeats remain interspersed between non-repeated sequences. Repeats of diverse sequences have been recorded in different organisms including *Drosophila* (Bruttlag and Peacock 1979) and man (Cooke and Hindley 1979). Though highly repeated sequences often remain associated with heterochromatin, their association with other repeated sequences is also not ruled out. High frequency repeats have been observed in telomeric heterochromatin (Appels *et al* 1978). Heterozygosity of the repeated DNA is associated with heteromorphicity in heterochromatin specially in out-breeders (Giraldez *et al* 1979; Jones and Flavell 1982). Long repeated DNA sequences have been located in maize, as well as shorter sites which are 150 nucleotides long (Lobov 1981). The heterochromatic knob in maize also has a very high content of repeated sequences (Rhodes *et al* 1981). In finger millets nearly 40–50 % of repeated DNA are interspersed with 18 % non-repeats (Gupta and Ranjekar 1981).

In *Scilla autumnalis* repeated sequences have been clearly demonstrated in B-chromosomes. In *Secale cereale* and *S. montana*, the same complement of repeated sequences is found in both. The respective amounts of 630 and 610 base pairs and distribution of 480 base pair families show substantial differences (Jones and Flavell 1982). In rye, on the other hand, each simple sub-repeat is interspersed by unrelated small sub-repeats. Even *in vitro* growth of some *Drosophila* cells leads to addition of several repeated sequences (Potter *et al* 1979). This is an example of conservatism and dispersion but simultaneously each taxonomic unit remains distinct.

Though usually moderate and minor repeats remain interspersed, in a few cases the repeats are not necessarily interspersed with non-repeats. In Syrian hamsters, short-repetitive sequences are organised in scramble-tandem clusters (Moyzis *et al* 1981) rather than individually, interspersed with non-repetitive regions. In rice genomes, such sequence interspersion has been noted in higher C_0t values. At lower C_0t values, however, DNA sequences have been interspersed within the repetitive fraction. All these facts clearly indicate that though minor and moderate repeats, which are undoubtedly amplified and dispersed, may sometimes not be associated with unique sequences, but in general interspersion has been recorded (Britten and Davidson 1971; Davidson and Britten 1979).

4.3 *Intervening sequences*

The most widely distributed repeated sequences in the eukaryotic genes are the *intervening sequences* or *introns* between coding elements. Such introns may be located at the terminal or initiation points or at intercalary positions (Crick 1979; Darnell 1978; Sogin *et al* 1977). The intervening sequences normally occur in multiple copies; they are transcribed but excised before translation. The introns for the genes for tyrosine, tryptophan and phenylalanine in yeasts are short repeats (Ogden *et al* 1979), whereas longer repeats are present in *Drosophila viridis* (Barnett and Rae 1979). In *Tetrahymena thermophila* such repeated sequences, the introns, in the ribosomal RNA genes are highly

conserved and have the same spliced junction as *Tetrahymena pigmentosa*. There is a high degree of homology between the intervening sequences of the two species. In *Drosophila melanogaster* the amplified dispersed copies belong to two categories—in one the sequences are related to each other and in the other these are not related (Glover 1977; Wallaver and Dawid 1978). However, such amplified intron-related sequences occur elsewhere in the genome as well (Dawid and Botchun 1977) and these have been suggested to be insertion elements.

4.4 *Transposons*

The idea of transposable (mobile) elements was first suggested by McClintock (1952), who visualized transposition of insertion sequences in maize. These sequences have a high degree of adjacent repeats, as in *Drosophila* and yeast. These are comparable to jumping genes (transposons). In interspecific hybrids of *Drosophila*, such transposition of mobile genetic elements has also been recorded. *In situ* hybridisation has led to the insertion of a small *mdg* element (jumping element) of *Drosophila viridis* to the 5th chromosome of *D. littoralis* (Evans *et al* 1982). This is a case of artificial transposition between different chromosomes and four such mobile dispersed genetic elements have been recorded for a specific family of repeats. There may even be 35 dispersed copies in a family of repeated sequences such as the ty-I gene in *Saccharomyces cereviscea*, which constitute about 2% of the genome. Tandem clusters of ty-1 genes have also been located and culturing the flies for a month has led to a detectable shift of their location in two strains (Cameron *et al* 1979). The transposable elements in yeast are quite remarkable in the sense that their movement is spontaneous and not activated by mutagens. In *Drosophila melanogaster* several families of such dispersed genes have been located, out of which *copia* are 5 kilobases long with direct repeats of 300 base pairs. The 412 sequences are each 7·3 kilobases with direct repeats of 500 base pairs (Potter *et al* 1979; Strobel *et al* 1979). In contrast to ty-1 of yeast which has a repetition of 258 base pairs presenting 100 copies of the gene, in *Drosophila* dispersed, amplified and mobile elements have also been noted in 297 and 225 base pair sequences but these are not so well defined. In view of the fact that interrelationships have been noted between transposable elements, a detailed study of their occurrence in several other dipterans has been suggested (Green 1980). Transposable elements have also been located in the human system (Calabretta *et al* 1983; Jacq *et al* 1979).

4.5 *Spacer*

Amplified sequences have been recorded as *spacers* in r-DNA of *Xenopus*, in *Drosophila* and in other organisms (Lewin 1980; Federof 1979; Jacq *et al* 1979). The spacer sequences have also been noted to be heterogenous and located in 5-histone gene clusters in mammals and other organisms (Cohen and Kedes 1979). In *Xenopus borealis* 5-H genes are contained in clusters, about 80 base pairs apart with spacers of variable length. Lately a new class of human interspersed repeated sequences different from the Abr family has been recorded, through a non-transcribed spacer group (r-DNA-NTS) of the mouse ribosomal gene, and utilised for screening a human gene library. It is a 250 base pair segment located between human S and B globin genes, mostly of tandem block of 17 TG dinucleotides. It is remarkable that homology of these spacer repeated sequences has been noted among *Xenopus*, pigeon, slime mold and yeast DNAs. This

homology in a wide spectrum of organisms indicates their highly conserved nature. Amplification and dispersion of such spacer sequences have been clearly demonstrated. In certain cases, the heterogeneity in size of the r-DNA repeating units is found to be due to the difference in length of the non-transcribed spacer regions (Waldron *et al* 1983). These differences have been taken to reflect the simple insertions into the non-transcribed spacer region of the major 'short' repeat. Evidently such insertions may also indicate the mobility of these sequences.

4.6 *Nucleotypic DNA*

In order to find a suitable explanation for the C-value paradox, the term *nucleotypic* DNA was coined (Bennet 1972; Evans and Rees 1971). This type of DNA is highly amplified and repeated in nature and is dispersed in its location in the genome. The word *nucleotypic* was used to account for its action related to properties of the nucleus, such as volume and duration of mitosis. A more detailed discussion is given later while dealing with the function of repeated sequences.

4.7 *Palindromes*

Palindromes or inverted repeats have been recorded at initiation and termination points as well as at other specific loci (Bostock 1980; Flavell 1980) and their ultrastructural and biochemical natures have been worked out. Leaving aside lower organisms (Klein and Welch 1980), genomes of several eukaryotes, like mammals, amphibia, insects and plants have been seen to possess inverted repeats (Bassi *et al* 1982; Deumling 1978; Hartman *et al* 1979; Stack and Comings 1979).

4.8 *Selfish* DNA

The terminology adopted for the amplified sequences is often unusual. *Selfish* DNA is applied to the repeated sequences which exist because of their capacity for duplication in a congenial cellular environment (Orgel and Crick 1980). No evolutionary function is visualized. This form of DNA may often be harmful to the phenotype, like the segregation-distortion locus in *Drosophila*. However, large portions of DNA in eukaryotic genes are of this type. The genes included are not only amplified and regulated to carry out the vital activities of the cell but their existence is also entirely dependent on their capacity to survive in the cellular environment.

5. Terminology

The selfish DNAs have been categorised differently by various authors and termed accordingly. Brown and Dover (1979) suggested that selfish DNAs are sequence-dependent and should be distinguished from sequence-independent ones, referred to as *ignorant* DNA. The latter possess the properties of dispersion and amplification with selfish DNA. The sharing of similar sequences, such as 5-S genes in different chromosomes, is a property of ignorant DNA, whereas *Copia* and Ty-I (transposons) are selfish in nature. Selfish DNA sequences, having the property of mobility and capable of transfer between different chromosomes, have been designated as *parasitic* to *symbiotic*

DNA (Dover and Doolittle 1980). The term *incidental* DNA has been applied to the amplified selfish DNA sequences taking into account their mode of origin (Jain 1980). It has been suggested that the pressure of mutation often leads to amplified sequences incidental to the mutation process, which may provide a steady pool of potential gene resources.

Some of the duplicated functional genes often become non-functional following mutation. Such sequences have been referred to as *pseudogenes* or *dead sequences* (Loomis 1973; Proudfoot 1980). These have been noted in different mammalian systems adjacent to the globin genes. The transcription of a pseudogene is responsible for the origin of human leucocyte interferon (Dawid *et al* 1981). In view of the importance of these sequences in certain cellular functions, the term pseudo or dead genes is not appropriate.

Excluding the unique sequences present in one or few copies which code for enzyme proteins, all other DNA sequences have several common properties. All the sequences so far mentioned show more or less common properties of amplification, dispersion and mobility. The term *dynamic* DNA was thus applied to cover all the epithets mentioned so far (Sharma 1978, 1981, 1983). Introns have been compared with transposons because of the common property of mobility which leads to their capacity for dispersion. In fact, selfish DNA includes introns and therefore all mobile sequences with the property of dispersion. Spacer sequences, as noted in 5-S genes, are considered to be ignorant and are amplified, dispersed and, possibly mobile. All the three properties have possibly been noted in Copia and Ty-I-sequence-dependent transposons. Parasitic and symbiotic DNA s are also amplified, dispersed and are capable of movement. Incidental DNA however just assumes that origin of such amplified sequences is incidental to mutation. Nucleotypic DNA is also repeated, dispersed and may have the property of movement. In general, amplification and dispersion are common to all types of DNA, whereas mobility has been demonstrated in some and not observed in others. The application of the term *dynamic* DNA takes into account all the properties attributed to such DNA sequences, which do not necessarily code for structural proteins, but have the capacity of amplification, dispersion and mobility. Accessory chromosomes, as the evidence so far presented shows, contain a high degree of repeated sequences. The extent to which these chromosomes represent the cytological embodiment of dynamic sequences is yet to be ascertained. Since the repeated sequences may later be converted into unique genes through mutations, these definitions are semantic in nature.

6. Origin

The origin of repeated sequences has been a matter of debate. Opinions differ with regard to the mechanisms of amplification. Saltatory replication has been widely suggested visualizing that eukaryotic organisms possess mechanisms for duplication of relatively short nucleotypic sequences (Cohen and Kaplan 1982). Unequal crossing over is a possible method of their origin (Smith 1976). Transposition of short repeats existing at the end of the transposons may also lead to their accumulation at a chromosomal site (Calos and Miller 1980). In any case, it is suggested that a small increase in the number of copies of a given short repeat may be a common event in the evolutionary time scale. In *Drosophila* too, transposition of the mobile elements has been regarded as responsible for the origin of specific repeats. In Syrian hamster short

or moderate repeats have been postulated to have originated as part of longer repetitive blocks. That is the reason why the majority of short repetitive regions in Syrian hamster DNA is in tandem clusters rather than as individuals interspersed with non-repeats (Moyzis *et al* 1981). It has been hypothesized that the high degree of repeats may represent selection for duplication of regulatory sequences. Such selections may involve deletions as well, leading to a decrease in DNA content (Jain 1980).

7. Function

The functional significance of repeated sequences is not yet fully understood. In a discussion of the various functions the basic fact remains that these are being tolerated during evolution. The evidence for their conservation has been recorded in various groups of organisms, including intragenic introns in *Tetrahymena* (Borschsenius *et al* 1981; Kan and Gall 1982), heterochromatic knobs in maize (Friedman *et al* 1982) and in primates, including man, gorilla and chimpanzee (Cooke *et al* 1982; Deininger and Schmidt 1976).

Notwithstanding the wide tolerance of such repeats, it has been suggested that their presence may not necessarily imply any functional significance (Doolittle and Sapienza 1980). In evolution, such sequences have been able to exist in a congenial environment for their multiplication with or without any effect on their phenotypes. On the other hand, their survival may even have a negative effect on certain phenotypes, like segregation-distortor locus in *Drosophila*. In order to account for the C-value paradox, it is suggested that there is no need to explore a function for every DNA sequence and a corresponding phenotypic selection.

This suggestion does not take into account the basic rationale in evolution where sequences are preserved against rigorous selection pressure. In addition, various functions are obviously related to amplified sequences. There has been an extensive change in DNA content during evolutionary advancement. Such variations in DNA content exist within and among species and may not always be related to the evolutionary progress of structural complexity of the organism. In reptiles, the species differences have been attributed to differences in DNA content (Ellison and Howard 1981). Both increase and decrease in the amount of DNA have been recorded. These changes have been explained on the basis of selection for duplication or deletion of regulatory sequences. In general, Price (1976) recorded a 15-fold range of DNA content per genome among angiosperms and a 2-4 fold variation within individual families. Interspecific variation, to some extent, has been correlated with geographical parameters, such as latitude. Among angiosperms more than a 100-fold range in DNA content has been reported and a 65-fold variation among the diploids. Even within a family of angiosperms, often a 40-fold increase in DNA content is observed. These variations, which often lead to C-value paradox, are mostly due to the acquisition or reduction of amplified DNA sequences, in addition to other factors. The correlation of amplified DNAs with cellular and organismic nature of evolutionary significance involves principally biophysical parameters, like volume of the nucleus and cell, chromosome size as well as minimum mitotic cycle, duration of meiosis and generation time. These parameters are associated with the evolution of annuals and perennials, where selective values of biophysical nature may have evolutionary indications.

Leaving aside such non-specific effects, the location of repeated sequences in higher

biological systems is rather diverse. The satellite DNAs have been noted to be attached to the ribosomal RNA in several organisms including the pea (Wall and Bryant 1981). They also occupy a non-random position in early embryos of *Drosophila viridis* (Ellison and Howard 1981). Such A-T rich sequences have been consistently found on the sites of the nuclei nearest to the vitelline membrane. Evidently a functional significance in terms of transport across the membrane is indicated, with an obvious role in a communication mechanism. The association of highly repeated sequences with heterochromatin and *vice-versa* is significant in this connection (Jones and Flavell 1982). The association of heterochromatic segments near the nuclear membrane was noted as early as 1949 by Vanderlyn (1949). He termed these regions liaison chromatin and assigned them a role in transport. The satellite DNA has also been considered to maintain structural integrity of the chromosomes by providing resistance to injury during their poleward movement and through recognition of homologous segments during recombination in meiosis. The heterogeneity of repeats has often been associated with heterozygosity, specially in relation to heterochromatin in outbreeders (Bedbrook *et al* 1980; Jones and Flavell 1982; Giraldez *et al* 1979; Lelley *et al* 1978; Singh and Robbelen 1979). The properties of transposition of such amplified sequences and their interspersion are considered to be of functional significance.

7.1 *Functions of interspersed repeats*

Various functions have been attributed to interspersed repeats (Davidson and Britten 1979). Their role in repair synthesis has been demonstrated in plant systems (Friedman *et al* 1982). Moderate repeats in *Lillium* have been shown to undergo specific repair synthesis during pachytene, through conservation of such DNA sequences termed as p-DNA (pachytene-DNA). It has been suggested that for families with high thermostability the lack of internal divergence is evidence for fairly recent introduction into a moderately repeated DNA class. p-DNA sequences, as a whole represent evolutionary ancient families—the products of strong selective pressure for an indispensible meiotic function. The heterochromatin in rye shows rearrangements where each sub-repeat is interspersed by unrelated non-subrepeats. The importance of such repeated sequences in the regulation of chromosome structure is indicated in their involvement in folding during pairing (John and Miklos 1979) as well as in differentiation of chromosome structure. Their presence at different loci in chromosomes in different strains of *Drosophila* may indicate a functional role (Beauchamp *et al* 1979; Young 1979). Similarly the increase in amplified sequences in cultured cells of *Drosophila* is also significant (Pottev *et al* 1979).

The importance of repeated sequences, specially in gene conversion and expression has been demonstrated in fission yeast (Egel 1981). Exchanges between repeated genes, leading to intergenic conversion and reappearance of suppressor active alleles, has become a powerful tool for genome exchange. The maintenance of sequence homogeneity of repeats is facilitated by such conversions (Klein and Petes 1981). In Asian and Caucasian human systems genetic exchanges occur within large repeated gene clusters of the α-globin region (Liebhader *et al* 1981). The best example for the control of repeats in the expression of non-repeats is provided in *Saccharomyces purpuratus* where RNA hybridisation has been noted preferentially with non-repetitive sequences linked with repeats (Lewin 1980).

In different organisms, sequences coding for histone, t-RNA and r-RNA are all repeats

of a different kind which can be transcribed. All the five genes coding for sea-urchin histone are repeated sequences having variable efficiencies of transcription (Henstschel and Birnsteil 1981).

An interesting case of gene expression is recorded in oocyte nuclei of *Xenopus* where injection of L-22 repeat sequences allows expression of the histone gene of sea-urchin (Grosschedl and Birnsteil 1980; Probst *et al* 1979). Various other functions have been attributed to moderate repeats including roles in evolution (Bachmann *et al* 1972), adaptive radiation (Stebbins 1972) and as a reservoir of new sequences (Hinegardner 1976). Sharma (1976, 1978) interpreted such sequences as loci for accumulation of tolerated mutations.

Inverted repeats or palindromes, in view of their occurrence in clusters at specific loci, have been assigned certain functions (Cavalier-Smith 1980). The term *fold back* or *snapback* DNA has been used and involvement in the formation of micronuclear DNA in ciliates has been suggested. Polymorphisms in inverted repeats are common and may have an evolutionary importance. Several functions have been attributed to palindromes. Their role in DNA replication at chromosome ends has been suggested by Cavalier–Smith (1976) and Heumanns (1976). Hartman *et al* (1979) have ascribed more than one function to such sequences.

Palindromic and repetitive sequences are intensely replicated under specified conditions (Borschsenius *et al* 1981). Initiation of replication involves certain parts which are comparatively long palindromic identical or very similar in the nucleotide sequences. Synchrony of replicon activation can be induced under specific conditions. Another extreme view has been taken by Deininger and Schmidt (1976) who attribute no function to palindromes. In general, the role of such sequences in specific protein synthesis has been visualized at different levels (Gailit and Fredrickson 1981). They may serve as recognition and functional systems at the DNA and RNA levels involving deletion and translocation of the genetic material (Klein and Welch 1980). In messenger processing and protein synthesis, their roles involve different functions, including the recognition of cleavage sites, termination of transcription and binding of regulatory proteins (Jelinak 1977; Robertson *et al* 1977; Wallace and Kass 1974). Cavalier-Smith (1976) suggested a role in attachment of chromosomes to each other, implying possibly information transfer. Bassi *et al* (1982) noted a gradient of interspersion of palindromes in the genome, with their gradual increase in higher organisms. In the maintenance of chromosome orientation in interphase and nuclear structure, the significance of such sequences has been emphasized. All these data in relation to palindromes may suggest their multiple functions and the preservation of such specific sequences at distinct loci may indicate a selective adaptation.

7.2 *Location-specific functions*

The location of repeated sequences in the introns has been clearly demonstrated. Such intervening sequences, in multiple copies, are present in the primary transcript following transcription but are spliced off before translation. Their role in the processing of the messenger, its transport and translation is signified by their localisation at the intercalary, terminal and initiation points (Crick 1979; Darnell 1978; Murray and Holiday 1979). Their specific activity in the rearrangement of functional exons, leading to the synthesis of new transcripts, has been demonstrated by recombination with introns in hen lysozymes (Artymiuk *et al* 1981). In the human

globin gene as well, the expressed sequences contain intervening repeats involved in intergenic conversion (Slighton *et al* 1980). In fact the intervening sequences with high degree of repeats may play a very significant role in the synthesis of new translatable transcripts and variable structural proteins. Splicing, followed by rejoining of different exons, may lead to variable messengers if alternate sequences are introduced in a different order. The information content of different allied anti-bodies may thus be stored in a single gene sequence, which has varying exon combinations following intron excision. The importance of intervening segments with repeated sequences assumes added significance in terms of accelerating intragenic recombinations.

The location of repeats in transposons is difficult to reconcile as they have no apparent function (Tchurikov *et al* 1978). The mobility itself permits dispersion leaving aside accelerating accumulation of certain segments at specific loci. It is likely that such mobile sequences aid in promotion of genetic diversity. The survival of sequences is also influenced by transfer to an environment where they will be tolerated.

The presence of repeated DNA in spacer sequences has been studied extensively in different organisms (Jacq *et al* 1979; Kuehn and Arnheim 1983). Such sequences often form heterogenous clusters (Lewin 1980) and their function is indicated in all the 5 histone gene clusters of mammals and other organisms (Cohn and Kedes 1979). In *Xenopus borealis* the clusters contain 5-S genes, 80 base pairs apart, separated by spacers of variable length. *In vivo* experiments have indicated that initiation and termination of transcription are facilitated through such sequences. However, the presence of detectable dissimilar 5-S gene clusters in species of *Xenopus* may suggest that common control is exerted only by a few sequences. In mouse, non-transcribed spacer fragments contain 13 tandem copies of 13 base pair sub-repeating units. It has been postulated that regions of inverted repeats as well as large Poly-T tracts may be related to the high level of r-RNA synthesis.

Several functions have been attributed to such repeated sequences. The enlargement of membranes as a consequence of increase in nuclear volume, caused by amplified nucleotypic DNA, has a selective advantage in providing an increased surface for the formation of the large number of pores needed for transfers of genetically controlled products. In addition to time for enlargement of nuclear volume, generation time, duration of mitosis, meiosis and other non-specific functions have been assigned to amplified and dispersed sequences.

The term *dynamic DNA*, proposed to embrace all such amplified sequences having the capacity of amplification, dispersion and mobility, describes the functions of repeats in biological systems (Sharma 1981, 1983). It exerts a dynamic influence on several non-specific and vital functions of the cell including nuclear behaviour, cell volume and chromosome size. Dynamic DNA has a distinctive role in messenger processing, reshuffling of exons and maintenance of integrity of chromosome structure by providing padding at respective loci. Notwithstanding the fact that evolution is not anticipatory, multiple copies are to be regarded as a natural mechanism for a steady supply of potential genetic diversity. Absolute functional redundancy or triviality, whether at cellular or organismic level, can hardly be reconciled with adaptations against the rigors of selection. The mobility and dispersion at multiple sites may be strategies adopted by these amplified sequences to promote genetic diversity and as a measure against complete elimination in evolution.

In general, these repeated sequences with dynamic functions do not generally code for structural proteins. But they do play a role in the maintenance and functioning of

structural sequences. Similarly genes for non-specific functions in cells are subject to physiological influences. The properties of amplification, dispersion and mobility confer a certain amount of flexibility and a wide functional range and may be subject to physiological and extracellular influences. Therefore, these sequences, which are often heterogenous in nature as revealed by different restriction sites, exhibit complexity in structure and flexibility in behaviour—the two features acquired during evolution.

Accessory chromosomes in plants, as the evidence so far indicates, may contain a high proportion of repeated DNA, perhaps as the cytological embodiment of repeated sequences. The properties of amplification and dispersion are inherent and mobility can be assumed in view of the dispersive property. There has been considerable debate with regard to the function of the accessory chromosomes. Significant correlation between soil type, climate and accessory chromosomes has been recorded in different species of *Festuca, Phleum* and *Centaurea* (Müntzing 1977). Adaptability of B-chromosomes in temperate and alpine climates has been clearly demonstrated in *Allium stracheyi* and species of *Arisaema* (Sau *et al* 1980; Sharma 1980, 1982, Sharma and Aiyangar 1961). The effects of crossover are noted in interspecific hybrids of *Aegilops, Lolium, Secale* and some insects (Jones 1975; Jones and Flavell 1982; John 1983). The accessory chromosomes certainly confer a buffering effect on the adaptability of a species and a threshold point over which a negative influence may be recorded.

8. Evolutionary significance

Evolutionary significance of repeated sequence is still a problem unsolved. The magnitude of the problem is manifested in the C-value paradox so well-known in biological systems. The examples quoted are those of lilies and amaryllids which contain nearly 20–30 pg of DNA as compared to nearly 1/10th of the amount in mammals. In other words, the fallacy lies between the information content and DNA content. The paradoxical-values are recorded even amongst the same groups of families of plants which do not differ significantly with respect to the complexity of their differentiation, *e.g.* within the genus *Vicia*, the two species *V. faba* and *V. sativa* differ greatly in the amount of DNA per nucleus.

Such major differences, even among related species which have consistency in range and restricted composition of repetitive to non-repetitive base sequences, have been classed as supplementary DNA by Hutchinson *et al* (1980). According to them, the restriction is possibly imposed by natural selection and DNA fractions of particular composition and organisation have specific ranges of tolerance. However, the non-repetitive sequences might have arisen through mutation of repeats and may not necessarily represent coding sequences but operative ones (John 1983).

Britten and Davidson (1971) suggested that evolution of a genome occurs through accumulation of redundant sequences which may later become active, after mutation or positioning in genetic regulation. It has, however, been proved by different authors that only a fraction of the total amount of DNA codes for structural proteins. As such the accumulation of repeated sequences is not necessarily associated with the progressive complexity of structure in an organism. However, if a regulatory role is also to be assigned to the so-called repeated DNA sequences in addition to non-specific functions, its quantitative increase may have some relationship with increase in complexity.

Several annual plants have lower DNA content than perennials. The latter, due to their longer life cycle, may have to cope with varied influences of the environment. As a result they may require greater number of regulatory sequences *vis-a-vis* higher DNA values (Price 1976). It has been claimed that low DNA content annuals have low flexibility as compared to the high evolutionary potential of a genome with moderate amount of redundant DNA.

Any discussion of DNA values in different groups of organisms must take into consideration the difference in cellular DNA content as seen in rodents (Patton and Sherwood 1982). The C-value variability of natural populations at the intraspecific level is not fully explored. The evolutionary significance and information content of variability of the amount of DNA major fractions which consist of repetitive sequences must await a complete analysis at intraspecific and functional levels.

The increase in the regulatory sequences concomitant with increase in phenotypic and physiological complexity requires a full compromise with the biophysical parameters like cell size, nuclear volume, generation time and periodicity (Price 1976). All these factors, taken together, must ultimately have a value over which the effect will either be negligible or injurious. Such a situation may explain a moderate DNA content with moderate number of repetitive units. An understanding is essential of the amount of information content and the DNA content per cell.

The increase in histological and biochemical complexity must necessarily involve a quantitative and qualitative increase in enzyme activity. Both of these would require high as well as continued transcriptional and translational capacity. The regulatory sequences may play a very important role in repression and derepression. In order to meet this role, especially during differentiation, additional amount of DNA is necessary to meet the transcriptional needs. The need for such an additional amount of DNA, whether for non-specific or regulatory function, may be met through a phylogenetic increase, *i.e.*, enhancement in the amount of repetitive DNA or polyploidy often associated with gene mutations.

With the gradual progress of evolution and the genetically controlled predetermined symmetry of an organ in a complex differentiated system, a heavy increase in the amount of DNA may not be conducive for growth and development. This would require greater cell volume, cell size, generation time, periodicity and other associated biophysical parameters. In order to circumvent these problems, an ontogenetic increase in DNA, involving endomitotic replication has been selected as a strategy in evolution. Such an ontogenetic increase is under genetic control and may involve whole or part of the genome, depending on the need for differentiation. With the provision for such an ontogenetic increase, the need for phylogenetic increment of DNA is greatly minimised, after an optimal level of DNA is reached. This is possibly the reason why in highly developed systems, in the hierarchy of evolution, the enhancement of DNA content, specially of the repeated sequences, is not as high as may be expected on the basis of the evidence from lower groups of plants and animals. With a moderate amount of repetitive DNA in the genome, which has the properties of regulation and control of certain biophysical parameters, the organism has developed a genetic system of ontogenetic increment through endoreduplication of DNA to meet the need for additional genetic elements during differentiation. The evolution of intron system in higher organisms, which consists mostly of repetitive elements, plays a very significant role in the reshuffling of exons to satisfy the requirements of gene products. The progress in evolution has therefore no doubt been associated with an increment of

amplified, dispersed and mobile DNA elements, dynamic in their properties only to a certain level. To secure maximum efficiency in evolution and optimize requirements in metabolism, there has been a distribution of these dynamic sequences at certain specific loci which play a very distinctive role in regulation and various non-specific but vital functions. Similarly mutations lead to the acquisition of the capacity of ontogenetic increase of DNA sequences. Experimental approaches to validate such a mechanism of evolution may solve the problem of additional genetic elements, at present an enigma in biology.

Acknowledgements

The author is grateful to Miss Sarmistha Sen and Miss Patralika Banerjee for assistance in preparing the manuscript and to Shri Jag Mohan Gupta and Shri Swapan Sur for typing the manuscript.

References

Angerur R C, Davidson E and Britten R J 1976 Single copy DNA and structural gene relationship among four sea urchin species; *Chromosoma (Berl)* **56** 213

Appels R, Driscoll C and Peacock W J 1978 Heterochromatin and highly repeated DNA sequences in Rye (*Secale cereale*); *Chromosoma (Berl)* **70** 67

Artymiuk P J, Blake C C F and Dippel A E 1981 Genes pieced together—exons delineate homologous structures of diverged lysozymes; *Nature (London)* **290** 287

Bachmann K, Goin O B and Goin C J 1972 Nuclear DNA amounts in vertebrates; *Brookhaven Symp. Biol.* **23** 419

Banerjee M and Sharma A K 1979 Variation in DNA content; *Experientia* **35** 42

Barnett T and Rae P M M 1979 A 9·6 kg intervening sequence in *D. viridis* r DNA and sequence homology in r DNA interruptions in diverse species of *Drosophila* and other Diptera, *Cell* **16** 763

Bassi P, Cremonini R and Cionini P G 1982 Cytological localization of inverted repeated DNA sequences in *Vicia faba; Chromosoma (Berl.)* **85** 453

Beauchamp R S, Mitchell A R, Buckland R A and Bostock C J 1979 Specific arrangements of human satellite *III* DNA sequences in human chromosomes; *Chromosoma* **71** 153

Bedbrook J R, Jones J, O'Dell M, Thompson R and Flavell R B 1980 A molecular description of telomeric heterochromatin in *Secale* species; *Cell* **19** 545

Bennet M C 1972 Nuclear DNA content and minimum generation time in herbaceous plants; *Proc. R. Soc. (London)* **B181** 109

Borschsenius S N, Merki Ina N A, Romanov G A and Vanvshin B F 1981 Palindromic and repetitive sequences in initiation sites of DNA replication in *Tetrahymena; Mol. Biol. (Moscow)* **15** 816

Bostock C J 1980 The organisation of DNA sequences in chromosomes, in *Cell biology* (ed) R Goldstein and D M Prescott (New York: Academic Press) **3** 1

Bostock C J, Gosden J R and Mitchell A R 1978 Localization of a male-specific DNA fragment to a sub-region of human Y-chromosome; *Nature (London)* **272** 324

Britten R J and Davidson E H 1971 Repetitive and non-repetitive DNA sequences and a speculation on the origins of evolutionary novelty; *Q. Rev. Biol.* **46** 111

Brown S D M and Dover G H 1979 *Nucleic Acid Res.* **8** 781

Bruttlag D and Peacock W J 1979 Sequences of the 1·672 g/cm 3 satellite DNA of *Drosophila melanogaster; J. Molec. Biol.* **135** 565

Calabretta B, Robertson D L, Barrera-Saldona H A, Lambrou T P and Saunders G F 1983 Genome instability in a region of human DNA enriched in Alu repeat sequences; *Nature (London)* **216** 219

Calos M P and Millers J H 1980 Transposable elements; *Cell* **20** 579

Cameron J R, Loh E Y and Davis R W 1979 Evidence of transposition of dispersed repetitive DNA families in yeast; *Cell* **16** 739

Cavalier-Smith T 1976 Long palindromes in eukaryotic DNA; *Nature (London)* **262** 255

Cavalier-Smith T 1980 How selfish is DNA?; *Nature (London)* **285** 617

Cohen E H and Kaplan G C 1982 Analysis of DNAs from two species of the viridis group of Drosophila and implications on satellite DNA evolution; *Chromosoma (Berl.)* **87** 519

Cohn R H and Kedes L H 1979 Non-allelic histone gene clusters of individual sea urchins (*Lytechinus pictus*) polarity and gene organisation; *Cell* **18** 843

Cooke H J and Hindley J 1979 Cloning of human satellite *III*: different components are on different chromosomes; *Nucleic Acid Res.* **6** 3177

Cooke H J, Schmidtke J and Gosden R R 1982 Characterisation of human Y-chromosome repeated sequence and related sequences in higher primates; *Chromosoma (Berl.)* **87** 491

Cremonini R, Ioro M L and Duranti M 1981 Differentiation of metaxylem cell line in the root of *Allium cepa* II. Changes in nucleotide sequence redundancy; *Protoplasma* **108** 1

Crick F 1979 Split genes and RNA splicing; *Science* **204** 264

Darnell J E 1978 Implications of RNA-RNA splicing in evolution of eukaryotic cells; *Science* **202** 1257

Davidson E H and Britten R J 1979 Regulation of gene expression: possible role of repetitive sequences; *Science* **204** 1052

Davidson E H, Jacobs H T and Britten R J 1983 Eukaryotic gene expression very short repeats and coordinate induction of genes; *Nature (London)* **301** 468

Dawid I B and Botchun P 1977 Sequences homologous to ribosomal insertions occur in the *Drosophila* genome outside the nucleolar organiser; *Proc. Nat. Acad. Sci. (USA)* **74** 4233

Dawid I B, Long E O, Di Novera P P and Pardue M L 1981 Ribosomal insertion like elements in *Drosophila melanogaster* are interspersed with mobile sequences; *Cell* **25** 399

Deininger P L and Schmidt C W 1976 An electron microscope study of the DNA sequence organisation of the human genome; *J. Molec. Biol.* **106** 773

Deumling B 1978 Localization of fold back DNA sequences in the nuclei and chromosomes of *Scilla, Secale* and Mouse, *Nucleic Acid Res.* **5** 3589

Doolittle W F and Sapienza C 1980 Selfish genes, the phenotype paradigm and genome evolution; *Nature (London)* **284** 601

Dover G and Doolittle W F 1980 Modes of genome evolution; *Nature (London)* **288** 646

Egel R 1981 Intergenic conversion and reiterated genes; *Nature (London)* **290** 20

Ellison J R and Howard G C 1981 Nonrandom position of the A-T rich DNA sequences in early embryos of *Drosophila viridis; Chromosoma (Berl.)* **83** 555

Evans G M and Rees H 1971 Mitotic cycles in dicotyledons and monocotyledons; *Nature (London)* **233** 350

Evans L S and Van't Hof J 1975 Is polyploidy necessary for tissue differentiation in higher plants; *Am. J. Bot.* **62** 1060

Evans M B, Yeninolopov G N, Peunova N I and Ityin Y 1982 Transposition of mobile genetic elements in the interspecific hybrids in *Drosophila; Chromosoma (Berl.)* **85** 375

Fedorof N V 1979 On spacers; *Cell* **16** 697

Flavell R B 1980 Molecular changes in chromosomal DNA organisation and origins of phenotypic variation; *Chromosomes Today* 7 42 (London: Oliver and Boyd)

Friedman B E, Bouchard R A and Stern H 1982 DNA sequences repaired at pachytene exhibit strong homology among distantly relation higher plants; *Chromosoma (Berl.)* **87** 409

Gailit J O and Fredrickson D W 1981 Possible palindromic DNA; *J. Theor. Biol.* **88** 241

Giraldez R, Cermemo M C and Orellana J 1979 Comparison of C-banding pattern in the chromosomes of inbred lines and open pollinated varieties of rye; *Z. pflanzenzuecht.* **83** 40

Glover D M 1977 Cloned segment of *Drosophila melanogaster* r DNA containing new types of sequence insertion; *Proc. Natl. Acad. Sci. (USA)* **74** 4932

Gosden J R, Lowrie S S and Cooke H J 1981 A cloned repeated DNA sequence in human chromosome heteromorphisms; *Cytogenet. Cell Genet.* **29** 32

Gosden J R, Lawrie S S and Gosden C M 1981 Satellite DNA sequences in human acrocentric chromosomes. Informations from translocations and heteromorphisms; *Am. J. Human Genet.* **33** 243

Green M M 1980 Transposable elements in *Drosophila* and other Diptera; *Annu. Rev. Genet.* **14** 104

Grosschedl R and Birnsteil M L 1980 Identification of regulatory sequences in the prelude sequences of H2A histone gene by the study of specific deletion mutants *in vivo; Proc. Nat. Acad. Sci. (USA)* **77** 1432

Gupta V S and Ranjekar P K 1981 DNA sequence organisation in finger millet; *J. Biosci.* **3** 417

Hartman N, Bell A J and McLanchlan A 1979 Organisation of inverted repeat sequences in hamster cell nuclear DNA; *Biochim. Biophys. Acta.* **564** 372

Henstschel C H and Birnsteil M U 1981 The organisation and expression of histone gene families; *Cell* **25** 301

Heumanns J M 1976 A model of replication of the ends of linear chromosomes; *Nucleic Acids Res.* **3** 3167

Hinegardner R 1976 Evolution of genome size, in *Molecular evolution* (ed) F J Ayala (Sunderland (Mass): Sinauer Press)

Hutchinson J, Narayan R K J and Rees H 1980 Constraints upon the composition of supplementary DNA; *Chromosoma (Berl.)* **78** 127

Jacq B, Jourdan R and Jordan B R 1979 Structure and processing of precursor 55 RNA in *Drosophila melanogaster; J. Molec. Biol.* **177** 785

Jain H K 1980 Incidental DNA; *Nature (London)* **288** 647

Jelinek W 1977 Specific nucleotide sequences in Hela cell inverted repeated DNA. Enrichment for sequences found in double stranded regions of heterogenous nuclear RNA: *J. Molec. Biol.* **115** 591

John B 1983 The role of chromosome changes in the evolution of orthopteroid insects in *Chromosomes in evolution of eukaryotic groups* (eds) A K Sharma and A Sharma **1** 1 (CRC Press)

John B and Miklos C L G 1979 Functional aspects of satellite DNA and heterochromatin; *Int. Rev. Cytol.* **58** 114

Jones J D G and Flavell R B 1982 The structure amount and chromosomal localization of defined DNA sequences in species of the genus *Secale; Chromosoma (Berl.)* **86** 613

Jones R N 1975 B-chromosome system in flowering plant and animal species; *Int. Rev. Cytol.* **40** 1

Kan N C and Gall J G 1982 The intervening sequence of the ribosomal RNA gene is highly conserved between two *Tetrahymina* species; *Nucleic Acid Res.* **10** 2809

Kimura M 1968 Evolutionary rate at the molecular level; *Nature (London)* **217** 624

Klein H L and Petes T D 1981 Intrachromosomal gene conversion in yeast; *Nature (London)* **289** 144

Klein H L and Welch S K 1980 Inverted repeated sequences in yeast nuclear DNA; *Nucleic Acid Res.* **8** 4651

Kuehn N and Arnheim N 1983 Nucleotide sequence of the genetically labile repeated elements 5 to the origin of mouse r DNA transcription, *Nucleic Acid Res.* **11** 211

Kunz W, Grimm C and Frauz G 1982 Amplification and synthesis of r DNA *The cell nucleus* (eds) H Busch and L Rothblum (New York; Academic Press) **C12** 156

Kurnitt D M 1979 Satellite DNA and heterochromatin variants: the case of unequal mitotic crossover; *Human Genet.* **47** 169

Lelley T, Josifek K and Kaltsikes P J 1978 Polymorphism in the giemsa C-banding pattern of rye chromosomes; *Can. J. Genet. Cytol.* **20** 307

Lewin B 1980 *Gene expression-eukaryotic chromosomes* (New York: Wiley Interscience) **2** 1

Liebhader S A, Gossens M and Kan Y W 1981 Homology and concerted evolution at the 1 and 2 loci of human B-globin; *Nature (London)* **290** 26

Lobov V P 1981 Repeated sequences in maize DNA; *Fiziol. Biochim. Kult. Rast.* **13** 477

Loomis W 1973 *Dev. Biol.* **30** 3

McClintock B 1952 Chromosome organisation and genic expression; *Cold Spring Harb. Symp. Quant. Biol.* **16** 13

Miesfield R, Krystal M and Arnheim N 1981 A member of a new repeated sequence family which is conserved throughout eukaryotic evolution if found between the human sand B-globin genes; *Nucleic Acid Res.* **9** 5931

Moyzis D K, Bonnet J, Li D W and Ts'o P O P 1981 An alternative view of mammalian DNA sequence organisation: *J. Molec. Biol.* **53** 871

Muntzing A 1977 Accessory chromosomes; *Ann. Rev. Genet.* **8** 243

Murray V and Holliday R 1979 *FEBS Lett.* **106** 5

Nagl W 1972 Molecular and structural aspects of the endomitotic chromosome cycle in angiosperms in *Chromosome today* (eds) C D Darlington and K R Lewis (London: Oliver and Boyd) **3** 17

Ogden R C, Beckmann J S, Abelson J, Kang H S, Soll D and Schmidt O 1979 *In vitro* transcription and processing of a yeast t-RNA gene containing an intervening sequence; *Cell* **17** 399

Olmo E 1981 Evolution of genome size and DNA base composition in reptiles; *Genetica* **57** 39

Orgel L E and Crick F H C 1980 Selfish DNA – the ultimate parasite; *Nature (London)* **284** 604

Patton J L and Sherwood S W 1982 Genome evolution in pocket gophers (genus *Thomomyces*) *II; Chromosoma (Berl.)*, **85** 163

Potter S S, Brorein W J, Dunsmuir P and Rubin G M 1979 Transposition of elements of the 412, copea and 297 dispersed repeated gene families in *Drosophila; Cell* **17** 415

Price H J 1976 Evolution of DNA contents in higher plants; *Bot. Rev.* **42** 27

Probst E, Kressmann A and Birnsteil M L 1979 Expression of sea urchin histone genes in the oocyte of *Xenopus laevis; J. Molec. Biol.* **135** 705

Proudfoot N 1980 Pseudogenes; *Nature (London)* **286** 840
Rhoades M M, Peacock W J, Dennis E S and Prior A J 1981 Highly repeated DNA sequence limited to knob heterochromatin in maize; *Proc. Natl. Acad. Sci. (USA)* **78** 4490
Robertson H, Dickson E and Jelinek W 1977 Determination of nucleotide sequences from double stranded regions of HeLa cell nuclear RNA; *J. Molec. Biol.* **115** 571
Sau H, Sharma A K and Choudhuri R K 1980 DNA, RNA and protein content of isolated nuclei from different plant organs; *Indian J. Exp. Biol.* **18** 1519
Sekerka V 1981 Changes in DNA content of interkinetic nuclei of epicotyl cells of *Vicia faba* L. during the process of differentiation; *Biologia* **36** 741
Sharma A K 1976 A new look at chromosome and its evolution; *Proc. Indian Natl. Sci. Acad. (Anim. Sci.)* (Silver Jubilee Medal Mem. Lecture) **42** 12
Sharma A K 1978a Change in chromosome concept; *Proc. Indian Acad. Sci. (Anim. Sci.)* **87** 161
Sharma A K 1978b Additional genetic elements in chromosomes; *Nucleus* **21** 113
Sharma A K 1980 Chromosomes and distribution of monocotyledons in the Eastern Himalayas, in *Tropical botany* (eds) K Larsen and L B Holm-Nilsen (New York: Academic Press).
Sharma A K 1981 DNA repeats and their terminology; *Nucleus* **24** 87
Sharma A K 1982 Genetic diversity of some monocotyledonous crops and their wild relatives in the Eastern Himalayan Region; *Workshop Conf. Trop. Pl. Res. SE Asia*, New Delhi
Sharma A K 1983 Additional genetic materials in chromosomes *Proc. Second. Chrom. Conf. Kew* (eds) P E Brandham and M D Bennett (London: George Allen and Unwin) **35**
Sharma A K and Aiyangar H R 1961 Occurrence of B-*chromosome* in, diploid *Allium strachevii* and their elimination, *Chromosoma* **12** 310
Sharma A K and Mookerjea A 1954 Induction of division in cells—a study of the causal factors involved; *Bull. Bot. Soc. G. C. Bose Mem.* Vol. 824
Sharma A K and Sharma A 1980 *Chromosome techniques—theory and practice*, 3rd edn (London: Butterworths)
Sherwood S W and Patton J L 1982 Genome evolution in pocket gophers (genus *Thomomyces*) *II, Chromosoma (Berl.)* **85** 163
Singh R J and Robbelen G 1979 Identification by giemsa technique of the translocations separating cultivated rye from the three wild species of *Secale; Chromosoma (Berl.)* **59** 217
Slighton J L, Blechl A E and Smithies O 1980 Human fetal Gr and Ar globin genes: complete nucleotide sequences suggest that DNA can be exchanged between these duplicated genes; *Cell* **21** 627
Smith G P 1976 Evolution of repeated DNA sequences by unequal crossing over; *Science* **191** 528
Sogin H L, Pace B and Pace N R 1977 Partial purification and properties of a ribosomal RNA maturation endonuclease from *Bacillus subtilis; J. Biol. Chem.* **252** 1350
Stack S M and Comings D E 1979 The chromosomes and DNA of *Allium cepa; Chromosoma (Berl.)* **70** 161
Stebbins G L 1972 In *Taxonomy, cytogenetics and evolution* (ed) D H J Valentine (New York: Academic Press)
Strobel S W, Dunsmuir P and Rubin G M 1979 Polymorphism in the chromosomal location elements of the 412, copea 297 dispersed repeated gene families in *Drosophila; Cell* **17** 429
Tchurikov N A, Ilyin Y V, Ananiev E V and Georgiev G P 1978 The properties of gene Dm 225, a representative of dispersed repetitive genes in *D. melanogaster; Nucleic Acid Res.* **5** 2169
Vanderlyn L 1949 The heterochromatin problem is cytogenetics as related to other branches of investigation; *Bot. Rev.* **15** 507
Waldron J, Dansmuir P and Bedbrook J 1983 Characterization of r DNA repeat unit in Mitchell *Petunia* genome; *Plant Molec. Biol.* **2** 57
Wall L V M and Bryant J A 1981 Isolation and preliminary characterization of cryptic satellite DNA in pea *Phytochemistry* **20** 1767
Wallace B and Kass T L 1974 On the structure of gene control regions; *Genetics* **77** 541
Wallaver P K and Dawid I B 1978 Ribosomal DNA in *Drosophila melanogaster II.* Heteroduplex mapping of cloned and uncloned r DNA; *J. Molec. Biol.* **126** 769
Young M W 1979 Middle repetitive DNA: a fluid component of the Drosophila genome; *Proc. Nat. Acad. Sci. (USA)* **76** 6274

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 505–523.
© Printed in India.

Strand plant communities of the Indian sub-continent

T ANANDA RAO and V M MEHER-HOMJI*

The Karnataka Association for the Advancement of Science, Central College, Bangalore 560 001, India

*Institut Français, Pondicherry, India

Abstract. Strand plant-communities, other than the mangroves, composed of psammophytes, halophytes have been described along with bioclimatic, edaphic factors for the seven coastal biogeographic sub-divisions of the sub-continent.

Keywords. Strand plant communities; psammophytes; halophytes; bioclimatic factors; edaphic factors.

1. Introduction

Coastal landscapes of the Indian sub-continent comprises of an array of ecosystems.

The wet coastal ecosystem or the mangrove swamps and the dry coastal ecosystems or strand or beaches are recognised on sight based combined physiognomic and topographic criteria. That these studies are not only of great theoretical but also of practical interest has been recently shown by detailed reviews (Rao 1971; Rao and Sastry 1972, 1974 a, b; Blasco 1975, 1977).

Since the mangals or mangroves have already been studied (Chapman 1977), we will review in this article the strand or beach plant-communities (other than the mangals) composed of psammophytes, facultative halophytes or salt tolerant glycophytes and also mention briefly the potential terrestrial vegetation in the immediate vicinity of the strand.

At the very outset it may be mentioned that the environmental terms 'dry' and 'wet' as applied to the coastal ecosystems appear rather conflicting. Mangrove which in a strict sense of the term cannot be treated as a 'wet' ecosystem because of the physiological dryness of the habitat, however, has been included in the volume on Wet Coastal Ecosystems.

Dry and wet categories are distinguished on the basis of the general climatic features like annual average rainfall and length of the dry season. Thus on one hand lie very dry stations like Pasni, Karachi, Bhuj, Dwarka in the northern extremity of the west coast with rainfall less than 500 mm and dryness for 10 to 11 months of the year. In the southern part of the west coast, Kozhikode (Calicut) and Trivandrum represent very wet conditions with very high rainfall (exceeding 3000 mm) and with only 3–4 months of dryness.

However, such a climatic division is rendered ineffective in view of the overwhelming edaphic influence of sandy and salty milieux in the immediate vicinity of the sea, imparting floristic and physiognomic homogeneity to the plant communities of the maritime strand. It is true that the plant formations beyond the confines of the maritime influence show close relationship with the macro-climatic features.

The maritime strand is that strip of coast extending from just above mean high water to the end of the sandy relief. This area is characterised by a maritime climate, high exposure to salt spray and shifting sand dunes. They are lithologically different and can be categorised under calcareous and non-calcareous zones with significant plant indicators. Rao and Sastry (1972) have attempted an improvement on the informal strand ecosystem classifications of the past workers. They have recognised three major types, namely strand sand, strand rock and strand coral based on combined physiognomic and topographic criteria. Further, these have been subdivided on the basis of zonation, dominant species and substrate features. This has revealed apparent zonation in some sectors and disturbed mosaics in others specially at the woodland stage mostly due to anthropogenic influences such as agricultural practices (paddy cultivation), raising of plantations of *Casuarina*, coconut and cashewnut in climatically humid zones.

2. Biogeographic sub-divisions

The coastline of the Indian sub-continent which extends for more than 6000 km may be divided into the following sub-divisions according to the climatic and general floristic features. Geologically, the entire coastline may be broadly placed under recent alluvium.

(i) *Coastal tract of Pakistan, Kutch and north-western part of Kathiawar forming eastern extremity of the Saharo-Sindian region of Eig (1931).* The climate as exemplified by the stations Karachi and Bhuj (figure 1) is arid with annual average rainfall of less than 500 mm and 9 to 11 months of dryness as per definition of Bagnouls and Gaussen (1953).

Natural vegetation in the sub-coastal plains is a thicket of *Prosopis cineraria* (L.) McBride-*Salvadora oleoides* Decne. type. The thicket aspect is given particularly by the bushes of *Euphorbia caducifolia* Haines which is the main species in the degraded areas where the soil is shallow and there are outcrops of rocks. It withstands the effect of salty winds.

(ii) *South-eastern Kathiawar and Gujarat north of the river Narmada may be included in the eastern half of the Sudano-Rajasthanian region of Meher-Homji (1965).* The climate of this tract is semi-arid with rainfall of 500–600 mm and dryness of 8 months. Ombrothermic diagram of the station Veralval in figure 1 illustrates this type of climate.

Thickets of *Acacia* spp. and *Capparis decidua* (Forsk.) Pax are encountered in the areas beyond the strand. The branching palm, *Hyphaene dichotoma* (White.) Furtado is dominant in the Delvada-Kodinar sector.

(iii) *Konkan coastal strip extending from southern Gujarat up to Goa.*

Climate varies from dry (*e.g.* Surat) to subhumid (Bombay) to humid type (Ratnagiri, figure 1).

The dry or moist deciduous forests with teak (*Tectona grandis* L.f.) form the potential natural vegetation beyond the maritime influence.

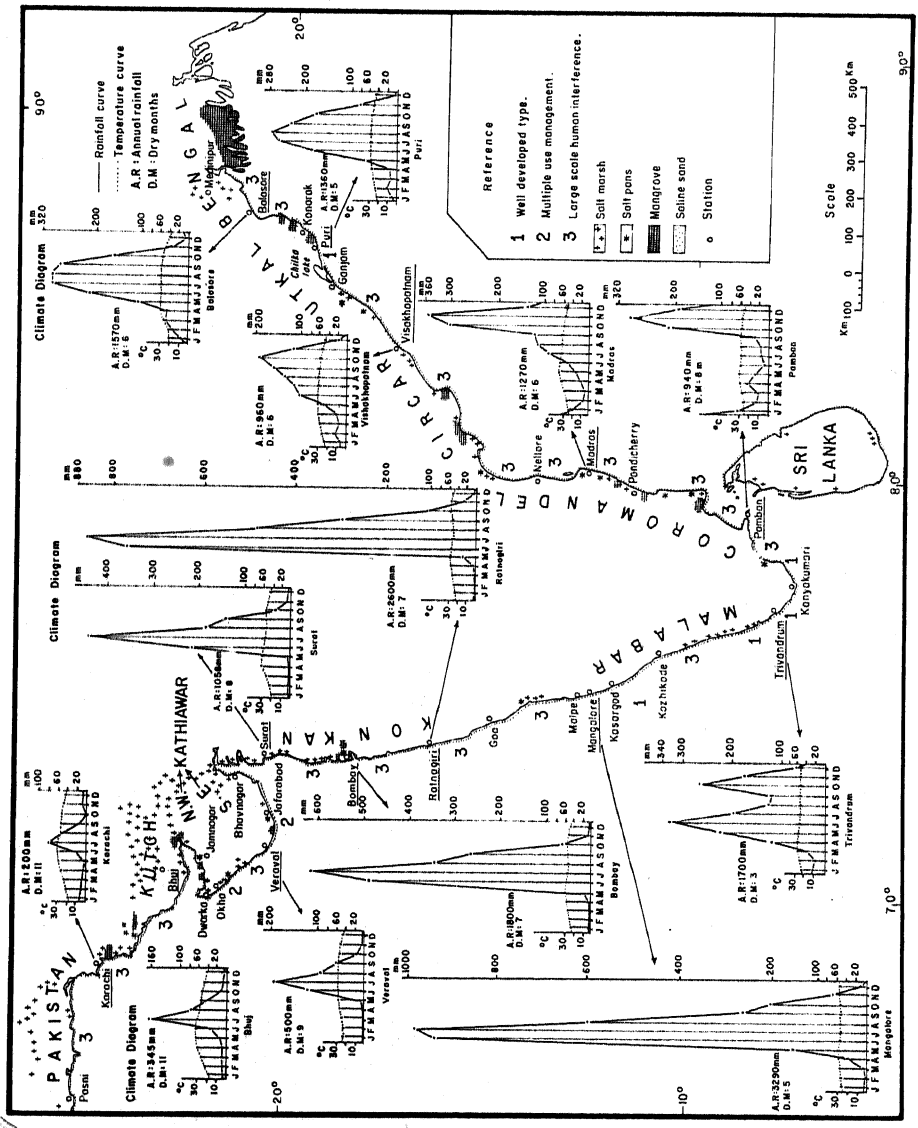


Figure 1. Coastal formations.

(iv) *Malabar region extending from Goa southwards up to Kanyakumari* (*Cape Comorin*). The climate is humid (Mangalore) to perhumid (Kozhikode and Trivandrum, figure 1).

The areas beyond the limit of the coastal sands have the potentiality to bear evergreen forests but through human interference the original forests are much degraded in the coastal tract and much of the area is brought under cultivation.

(v) *Coromandel-Circar coastal plains of Tamil Nadu and Andhra Pradesh of the South-eastern part of India*, characterised by tropical dissymetric regime of rains. South-west monsoon brings only light rains during June–September; heavy rains occur in October–November due to the depressions formed in the Bay of Bengal. Climate is dry (Pamban) to subhumid (Madras and Visakhapatnam, figure 1).

Potential vegetation of this tract is peculiar: dry evergreen forest (Champion 1936; Meher-Homji 1973, 1974; Blasco and Legris 1973; Sprangers and Balasubramanian, 1978; Werger and Sprangers 1982).

(vi) *The islands between India and Sri Lanka* though climatically similar to the Coromandel coast differ from the mainland in having coral sand and distinct vegetation (Rao *et al* 1963a, b).

(vii) *Utkal and Bengal coastal plains* The Utkal coast is the northern prolongation of the Circar region in Orissa state. It is formed of the Mahanadi delta and the coastal plains of Bhadrak, Balasore and Kunthi. The Chilka lake of Puri district which though only a few metres deep varies in size from 900–1200 km^2 and is alternately salty and fresh. The Medinipur coast marks the extension of the Utkal coast into Bengal.

The climate as exemplified by the stations of Puri and Balasore (figure 1) is humid.

The potential vegetation of the interior areas is typified by deciduous sal (*Shorea robusta* Gaertn.) forests. Along the sandy fringes, *Borassus flabellifer* L. and *Phoenix sylvestris* Roxb. are commonly met with.

Figure 1 shows the coastal habitats of the sub-continent. The strand sand type is dispersed on the sandy relief beyond the mean high tide limit. Zonation of the vegetation into an outer pioneer zone, a closed herbaceous zone, middle mixed or bushy zone and inner woodland zone can be made out (Rao and Sastry 1972).

The outer pioneer zone is characterised by the community of *Ipomoea pes-caprae* (L.) R. Br., *Spinifex littoreus* (Burm.f.) Merr., *Cyperus arenarius* Retz., *Canavalia maritima* (Aubl.) Thw. and *Launaea sarmentosa* (Willd.) Alston throughout the sub-continent.

3. Pakistan, Kutch, North-western Kathiawar

The coastal area may be divided into three sub-divisions: shore, coastal and sub-coastal plain (Rao and Aggarwal 1964).

(a) *Sandy strand flora*: The pioneer plant is *Ipomoea pes-caprae*, a strand creeper. Closely following it on the back shore in order of relative abundance are *Cyperus arenarius, Launaea sarmentosa, Borreria articularis* (L.f.) F. N. Will, and *Boerhavia diffusa* L. (figure 2).

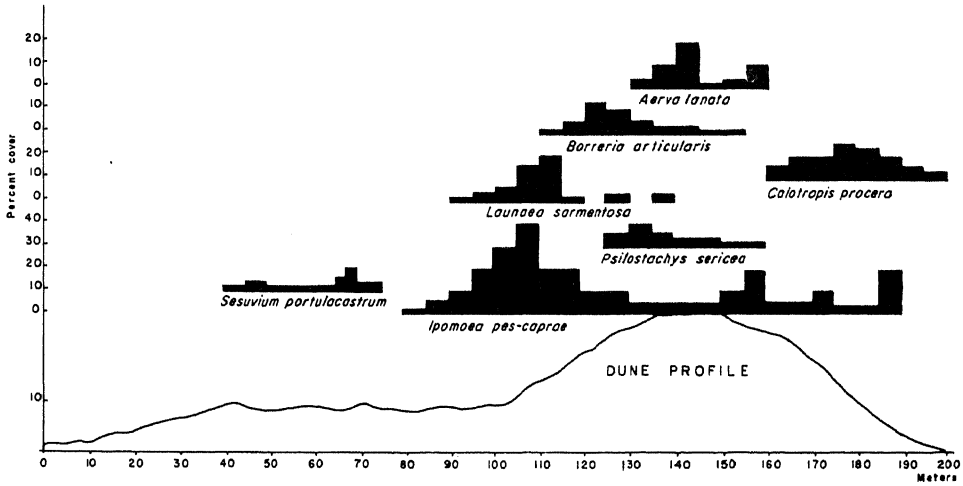


Figure 2. Percentage cover and zonal pattern of the strand/dune plants in a belt transect over a coastal strip, Kathiawar.

Near Dwarka and Okha, *Asparagus dumosus* Baker is a localised strand species.

Around Gharo village in the Indus delta (Pakistan), the sand and sand-dunes are covered with *Convolvulus scindicus* Stocks, *Leptadenia pyrotechnica* (Forsk.) Decne., *Launaea resedifolia* (L.) O. Ktze., *Aerva persica* (Burm.f.) Merrill and *Asparagus gharoensis* Blatter besides *Ipomoea pes-caprae, Launaea sarmentosa* and *Cyperus arenarius* (Blatter *et al* 1929).

Soils are sandy to sandy loamy in texture, moderately alkaline (pH 8–8·4), low in organic matter which ranges from 0·25–0·64 %. Calcium carbonate ranges from 13–92 %. Sodium chloride from 0·01–0·19 % (Rao and Shanware 1967).

(b) The *rocky* shore is described under the second region of Southern Kathiawar-Gujarat north of the Narmada.

(c) *Muddy shore*: This includes shallow depressed areas periodically flooded by tidal waters. Such areas develop mangroves with *Avicennia marina* Vierh. var. *acutissima* Stapf & Moldenke. A little further inland appear *Salt marshes* with *Aeluropus lagopoides* (L.) Trin. ex Thw., *Atriplex stocksii* Boiss., *Salicornia brachiata* Roxb., *Suaeda fruticosa* Forsk., *Urochondra setulosa* (Trin.) Hubbard and *Sesuvium portulacastrum* L.

Fringing this zone along moist, less saline soils are *Fimbristylis cymosa* R. Br., *Scirpus maritimus* L. and *Tamarix troupii* Hole.

The Ranns of Kutch

A large area of Kutch, mostly the northern and western parts, is saline marshy; this is the Great Rann of Kutch. The Little Rann lies in the south-east. These are vast expanses of mud flats flooded by the sea during the monsoon.

They do not support any vegetation. However, some halophytic grasses are found at their margins: *Aeluropus lagopoides* (L.) Trin. ex Thw., *Sporobolus helvolus* (Trin.) Th.

Dur. et Schinz, *S. marginatus* Hochst., *S. coromandelianus* (Retz.) Kunth, *S. maderaspatanus* Bor, *Urochondra setulosa, Cynodon dactylon* (L.) Pers., *Crypsis schoenoides* (L.) Lamk., *Eriochloa procera* (Retz.) Hubb., *Eragrostis ciliaris* (L.) R. Br., *Cenchrus ciliaris* L., *Dactyloctenium aegyptium* (L.) P. Beauv. and *Chloris virgata* Sw.

In the saline areas bordering the Ranns, plantations of *Prosopis juliflora* DC. have been raised.

Salt pans

Some of the saline marshes are transformed into salt pans. The common circum salt-pan plants are *Aeluropus lagopoides* (L.) Trin. ex Thw., *Clerodendrum multiflorum* (Burm.f.) O. Ktze., *Cressa cretica* L., *Sporobolus virginicus* (L.) Kunth, *Suaeda nudiflora* Moq. and *Tylophora indica* L.

4. Southern Kathiawar-Gujarat north of the Narmada river

Sandy beaches and sand-dunes are covered with *Ipomoea pes-caprae*, (L.) R. Br., *Launaea sarmentosa* (Willd.) Alston, *Evolvulus alsinoides* L., *Convolvulus arvensis* L., *Boerhavia diffusa* L. Thus the sandy strand flora is more or less similar to the one described for the preceding region though the presence of *Hydrophylax maritima* L. f. a pioneer strand plant, *Halopyrum mucronatum* (L.) Stapf and *Psilostachys sericea* (Koen. ex Roxb.) Hook.f. is worthy of notice.

Spinifex littoreus and *Cyperus pedunculatus* (R. Br.) Kern which disappear in this territory reappear in Southern Gujarat.

Rocky shore

The foreshore is free from sand but it is composed of a lime-stone reef of considerable width. The pot holes and crannies developed in the rocks are filled up with sand and bear *Atriplex stocksii* Boiss., *Fagonia cretica* L., *Polycarpea spicata* Wt. and Arn. and *Limonium stocksii* (Boiss.) O. Ktze. on the slopes. More species are added towards the summit of the rocks: *Helichrysum cutchicum* (Cl.) Rolla et Desh., *Enicostema hyssopifolium* (Willd.) Verd., *Kickxia ramosissima* (Willd.) Janchen, *Lindenbergia urticaefolia* Lehm., *Portulaca quadrifida* L., *Pulicaria angustifolium* DC., *Sporobolus diander* (Retz.) Beauv., among others.

Further inland is encountered rocky sandy strand with mingling of coastal and inland plants like *Aerva lanata* (L.) Juss., *Capparis cartilaginea* Deone., *Tephrosia purpurea* (L.) Pers.

Soils are sandy to loamy sand and at times sandy clay with pH ranging from 7·8–8·1. Organic matter is low: 0·43–0·72 %. Sodium chloride varies from 0·007–0·086 % and calcium carbonate from 23–69 %.

The branched palm *Hyphaene dichotoma* is encountered on the sandy coast near Diu, Delvadá and Kodinar sectors. Further southwards it occurs at Daman and Bombay. Thus the endemic occurrence of the species in the erstwhile Portugese territories is a feature worth noting. It may also be mentioned that *H. dichotoma* has close affinities with *H. thebaica* Mart. of Africa (Rao and Korlahalli 1969; Rolla 1963).

The presence of *Acacia planifrons* Wt. and Arn. in the vicinity of Porbandar is worth noting. It is also found after a long discontinuity in the semi-arid coastal areas of South

India, near Tuticorin and Pamban in Ramanathapuram, Tirunelveli districts. Morphologically, the species is closely allied to some African members of the genus *Acacia*, notably *A. tortilis* Hayne of north-east Africa and Aden.

The fact that there was an important trade in horses between Arabia, Africa and the Indian ports Porbandar and Tuticorin in the past may suggest that *A. planifrons* has been an accidental introduction in India, where over centuries it has emerged as a species only slightly distinct from the African members of the genus (Viart 1963; Meher-Homji 1970).

5. Konkan

The Konkan coast extends from South of the river Narmada to Goa. This coast is more or less cliffy. The northern part under riverine and tidal influence becomes muddy, otherwise the coast has jutting headlands and covered with a thick mantle of sand.

South of Daman there are marshy areas fringed with mangroves; raised grounds with less salinity bear saline pastures composed of *Aeluropus lagopoides, Fimbristylis junciformis* Vahl, *Paspalum vaginatum* Swartz. and *Sporobolus virginious* (L.) Kunth.

Newly recorded occurrence is that of *Psilostachys sericea* on sands near Bombay. Near Marmagoa *Cyperus pedunculatus, Zoysia matrella*, and *Perotis indica* at Daman are the other interesting plants. *Calophyllum inophyllum* L. is absent along the Gujarat coast.

Satyanarayan (1958) has recognised the following seven strand associations along the Bombay coastline.

(i) *Ipomoetum pes-caprae: Ipomoea pes-caprae* forms the pioneer zone on sandy beaches and is the dominant plant of this association. Associated species are *Alysicarpus vaginalis* DC. and *Portulaca oleracea* Linn.

Behind the pioneer zone lies the mature community of *I. pes-caprae*. Among the companion species occur *Corchorus aestuans* Linn., *Tridax procumbens* Linn., *Phyllanthus fruternus* Web., *Borreria stricta* Schum., *Launaea sarmentosa, Boerhavia diffusa* Linn., *Indigofera cordifolia* Heyne, *Crotalaria retusa* Linn., *Dactyloctenium sindicum* Boiss., *Leucas aspera* Spreng., *Opuntia elatior* Mill. *Urginea indica* Kunth is also recorded (Shah 1962). During monsoon *Chlorophytum tuberosum* Baker is a common plant on the sand.

Organic matter which is 0·05 % in pioneer zone increases to 0·16 % in the mature zone whereas the total soluble salts decrease from 2·32 % in the first zone to 1·24 % in the second.

Canavalia lineata DC., *C. ensiformis* DC. and *Sporobolus glaucifolius* Hochst. are other sea-shore plants. *Scaevola taccada* (Gaertn.) Roxb. has disappeared from Juhu beach but *Psilostachya sericea* is a new record for Bombay (Rao 1971).

(ii) *Spinificetum*: This association is restricted mostly to the island of Manori. It represents a stage higher than the pioneer *Ipomoea pes-caprae. Spinifex littoreus* is considered the characteristic exclusive species and *I. pes-caprae* the preferential species.

Among the companion species may be cited *Boerhavia diffusa* Linn., *Borreria stricta* Schum., *Cyperus rotundus* Linn., *Eragrostis ciliaris* Link., *Pedalium murex* Linn., *Launaea sarmentosa* and *Canavalia lineata* DC.

Soils are coarse sandy in texture, low in moisture (0·57–0·76 %) and organic matter (0·26–0·50 %). Soil reaction is alkaline (pH 7·9–8·1) but compared to the preceding association total soluble salts are much less (0·08–0·14 %).

Further inland this association is replaced by a shrub zone of *Pandanus tectorius* Soland.

(iii) *Pedalietum muricae*: This association is confined to the island of Arnala. The dominant plant is *Pedalium murex* Linn. which is also the characteristic exclusive species. *Perotis indica*, a typical sand strand grass, is considered as the characteristic preferential species. Companion species are *Borreria stricta, B. hispida* Schum., *Paspalum vaginatum* Sw., *Eragrostis ciliaris, Lindernia ciliata* Pennell and *Corchorus aestuans* Linn.

Higher moisture content, less alkaline reaction (pH 7–7·6), somewhat higher amounts of organic matter and predominantly fine sandy texture with higher amounts of clay distinguish the soils of this association from those of *Spinificetum.*

(iv) *Tephrosietum purpurae*: This herbaceous association marks a transition to the inland vegetation. The dominant species is *Tephrosia purpurea* Pers. The characteristic exclusive species is only one viz. *Eragrostis tremula* Hochst. Preferential species are *Alysicarpus vaginalis* var. *nummularifolius* Baker and *Sida veronicaefolia* Lam. The affinity with other psammophytic communities is indicated by species like *Perotis indica, Alysicarpus rugosus* DC., *Euphorbia hypericifolia* var. *parviflora* Hook., *Pedalium murex* Linn., *Leucas aspera* Spr., *Rungia parviflora* var. *pectinata* and others.

Compared to the preceding association, the soils of *Tephrosietum* show an increase in silt, clay, moisture and organic matter and a somewhat lesser pH (6·5–6·8). Percentage of total soluble salts remains the same but change in soil texture leads to increased cation exchange capacity.

Further inland is a shrub zone dominated either by *Alangium salvifolium* (L. f.) Wang. or *Calycopteris floribunda* Lam.

Cyperetum rotundi, Sesuvietum portulacastri and *Aeluropetum repensi* are salt marsh communities.

(v) *Cyperus rotundus*: association sometimes forms the first zone behind the mangroves. It also occurs in rocky crevices where there is accumulation of soil particles. The characteristic preferential species are *Sporobolus glaucifolius* Hochst., *Fimbristylis polythricoides* Vahl and *F. ferruginea* Vahl.

Among companion species may be mentioned *Leucas aspera* Spreng., *Launaea sarmentosa, Paspalum vaginatum* Sw., *Rungia parviflora* var. *pectinata, Lindernia ciliata* Pennell, *Aerva lanata* Juss.

Soils are sandy, strongly alkaline (pH 8·1–8·6) but poor in moisture and organic matter. Soluble salt content is not too high (0·10–0·23 %); where sea water bathes the vegetation at least 8 times a month, *Cyperus rotundus* makes room for *Fimbristylis ferruginea.*

(vi) *Sesuvietum portulacastri*: This community is as typical of tidal mud flats as *Ipomoea* is of sandy shores. It marks the beginning of the tidal zone. The dominant and characteristic species is *Sesuvium portulacastrum* Linn. Associated species are

Sporobolus virginicus Kunth., *S. glaucifolius* Hochst., *Paspalum vaginatum, Cressa cretica* Linn., *Vitis trifolia* Linn.

Silty loam in texture and strongly alkaline (pH 8·5–8·8), the soils are very rich in soluble salts (3·1–3·4 %) which explains the paucity of the species.

(vii) *Aeluropetum*: This 'salt meadow' community comes up either in areas from where the mangroves dominated by *Avicennia* are cut down or in the mud flats as a pioneer or yet on "bunds" along the salt pans.

The dominant and exclusive species is *Aeluropus lagopoides* while the preferential species are: *Sesuvium portulacastrum, Sporobolus glaucifolius, S. virginicus, Suaeda maritima* Dum., *Suaeda nudiflora* Moq., *S. fruticosa* Forsk., *Clerodendrum inerme* Gaertn., *Paspalum vaginatum, Fimbristylis polythricoides.*

The association occurs both in places subject to tidal influence and those above the influence of tides.

With reference to the soils of the preceding *Sesuvium* community, the soils of *Aeluropetum* are less saline with total soluble salts ranging from 2·2–2·5 %, mildly alkaline (pH 7·6–7·8), silty in texture and contain higher amounts of organic matter and moisture.

Succession

Ipomoea pes-caprae forms the pioneer stage of succession. This stage is succeeded by *Spinificetum* which in turn is replaced either by shrubby *Pandanus tectorius* or by *Pedalium murex* if moisture and organic matter are higher and pH lesser. The next higher successional stage is that of *Tephrosietum purpurae* leading to the shrubby zone of *Alangium salvifolium* or *Calycopteris floribunda.*

6. Malabar region

Two examples may be given from this region: (i) Along the Malpe coast in the Udipi taluk of South Kanara district, Karnataka State, the pioneer zone of *sandy strand* is occupied by *Ipomoea pes-caprae* and *Cyperus pedunculatus*, which are found growing in pure or mixed stands with a sparse growth of *Oldenlandia herbacea* and *Launaea sarmentosa* (Arora and Aggarwal 1965) (figure 3).

Coarse sands from the pioneer zone are moderately alkaline. The organic matter content is very low. Dissolved solids and sodium chloride content range between 0·130–0·145 % and 0·076–0·087 % respectively.

Beyond the outer exposed beaches where the sand slope decreases to provide a stretch of fairly level ground, *Ipomoea spinifex* association dominates with *Crotalaria nana* Burm., *Polycarpaea corymbosa* (L.) Lamk., *Borreria hispida, Fimbristylis barbata* (Rottb.) Benth. and *Acrocephalus indicus* (Burm.) O. Ktze.

The main strand zone is occasionally interrupted by *Scaevola taccada* (Gaertn.) Roxb., *Pandanus tectorius, Clerodendrum inerme* Gaertn. and *Derris trifoliata* Lour.

Soils from the main strand zone are loamy to sandy with moderate alkalinity. Their organic matter content is higher but dissolved solids and sodium chloride contents are lower than the soils of the pioneer zone.

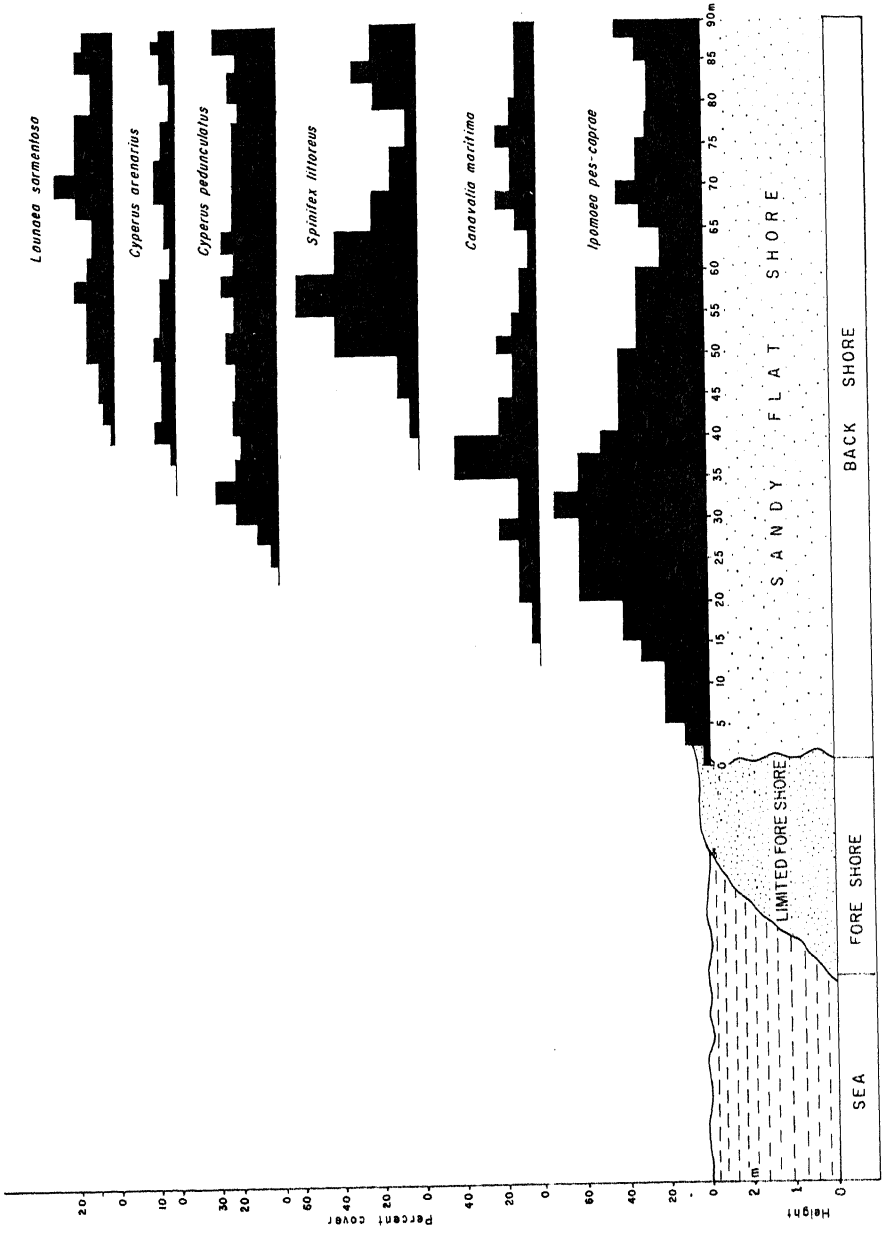


Figure 3. Percentage cover and zonal pattern in pioneer zone of Malabar coast.

Muddy shore under backwaters

Acanthus ilicifolius L. is the most common species covering muddy banks followed by thickets of *Clerodendrum inerme, Derris trifoliata* which in turn are followed by the mangroves.

Soils are loamy sand to sandy with mild alkalinity. Organic matter content, dissolved salts and sodium chloride are higher than in the preceding soils.

The transition from the herbaceous *Ipomoea pes-caprae* community to the littoral woodland or *Barringtonia speciosa* formation is not developed along the Malpe coast as the trees are cut down for growing coconut plantations. The belt of woody species may be split into two zones: one composed of *Scaevola taccada* and *Pandanus tectorius* generally growing on the seaward fringe and the other composed of tall species like *Barringtonia asiatica* (L.) Kurz, *Calophyllum inophyllum, Terminalia catappa* L., *Morinda citrifolia* L., *Hibiscus tiliaceus* L. (Arora and Aggarwal 1965).

Noteworthy strand plants of Karnataka part of Malabar region according to Rao (1971) are *Anotis carnosa, Crotalaria nana, Cyperus pedunculatus, Euphorbia atoto* Forst., *Indigofera aspalathoides* Vahl, *I. uniflora* Ham., *Ipomoea macrantha* Roem. & Schult. *Scaevola plumieri* occurs near Surthakal.

(i) The sandy vegetation of Veli (Trivandrum) is described by Thomas (1962).

Ipomoea pes-caprae and *I. repens* occur in great abundance along with *Launaea sarmentosa, Euphorbia rosea, Aerva lanata, Heliotropium scabrum* and *Zoysia matrella.*

Further inwards towards the land appears the community of *Catharanthus roseus* forming thick patches. Other common herbs on the sand are *Evolvulus alsinoides* L., *Rhynchelytrum repens* (Willd.) Hubb., *Cyanotis axillaris* R. & S., *Commelina nudiflora* L., *Polycarpaea corymbosa* (L.) Lamk.

The Kerala part of the Malabar region is treated in detail by Rao (1978). The interesting plants of the area are *Lumnitzera racemosa* Willd. near Quilon, *Premna serratifolia* L. and a salt tolerant fern *Acrostichum aureum.*

7. Coromandel-Circar region

A phytosociological study carried out in the Pondicherry region showed that the coastal belt is characterised by the alliance of *Catharanthus roseus* (L.) G. Don (Marlange and Meher-Homji 1965). Under this alliance are grouped three associations.

(i) *Spinifex littoreus* association develops on the sea shore and on the new dunes.

This association has three characteristic species: *S. littoreus, Cyperus arenarius* and *Polycarpon loeflingae* Bth. & MK. f. The former is gregarious and dominates the association. *Catharanthus roseus* is the species of alliance common to all the associations of the coastal zone. Once a common species, it has been heavily exploited since 1971 for its use as a medicinal plant and is presently rare on the coastal sands.

Launaea sarmentosa (Willd.) Alston is the *species of alliance* between the communities of saline soil and those of sandy littoral.

The following species seem to be indicators of sandy texture; they are common to the communities of the littoral sands and of the ferrallitic sandy loam: *Canscora diffusa* R. Br., *Allmania nodiflora* R. Br. var. *procumbens* Hk. f., *Waltheria indica* L., *Perotis indica* O. Ktz., *Tephrosia purpurea* Pers., *Mollugo disticha* Ser., *Gisekia pharnaceoides* L.,

Bulbostylis barbata (Rottb.) Clarke, *Polycarpaea corymbosa, Leucas suffruticosa* Benth.

(ii) On the older consolidated dunes, the association of *Spinifex* gives place to that of *Tephrosia purpurea* Pers. *Spinifex* and *Cyperus arenarius* disappear. *T. purpurea* becomes abundant and the general floristic composition remains the same as for the preceding association.

(iii) The association of *Catharanthus roseus* marks a more highly evolved stage of succession on the stabilised dunes. *Turnera ulmifolia* L. makes its appearance as a characteristic species of the association. Some more species of liaison of sandy littoral soils-ferrallitic sandy loam creep in: *Cleome aspera* Koen. ex DC., *Merremia tridentata* (L.) Hall. f., *Zornia gibbosa* Span., *Desmodium rottleri* Baker and *Evolvulus alsinoides* L.

Among the species of wider ecological amplitude which occur as companion species in this association, mention may be made of *Alysicarpus vaginalis* DC., *Euphorbia rosea* Retz., *Justicia prostrata* Gamb., and *Jatropha gossypifolia* L.

Saline soils: The association of *Suaeda nudiflora* occurs in the saline marshy zones in the Pondicherry region. The two exclusive species are *Suaeda nudiflora* Moq. and *S. monoica* Forsk. Among other characteristic species of this halophytic community mention may be made of *Cressa cretica* L., *Aeluropus lagopoides* and *Heliotropium curassavicum* L.

Launaea sarmentosa (Willd.) Alston and *Geniosporum tenuiflorum* (L.) Merr. are the two species common to the communities of saline marshes and coastal sands.

From Kanyakumari region, Nayar (1959) reports the following communities on the foreshore sand:

(a) *Spinifex littoreus—Cyperus arenarius* community, (b) *Ipomoea pes-caprae—Tephrosia purpurea* community, (c) *Calotropis—Cyperus* community.

Rao *et al* (1974) and Sebastine and Ellis (1967) have also listed the species from the Kanyakumari and the point Calimere coastal regions. The occurrence of *Myriostachys wightiana* HK. f. in the sandy saline flats around Point Calimere marks the Southern most limit of the species along the Indian coast (Rao 1971).

The Circar part of the Andhra Pradesh sandy coast is described by Sastry and Rao (1973).

8. The vegetation of the islands between India and Sri Lanka

This has been treated by Rao *et al* (1963a, b), Stoddart and Fosberg (1972), Sundararaj and Nagarajan (1964) and Srinivasan (1960).

The island areas are characterised by strand coral habitats. The coral strand is made up of weathered coral reef, foraminiferal sand and shell deposits. The vegetation of the pioneer zone is composed of *Pemphis acidula* Forst., *Suriana maritima* L., *Cordia subcordata* Lamk. and *Avicennia officinalis* L. The raised coral ridges support *Dodonaea viscosa* Jacq, *Halopyrum mucronatum* Stapf, *Excoecaria agallocha* L., *Thespesia populnea* Corr., *Lumnitzera racemosa* Willd. and *Scaevola* spp.

Newly recorded occurrence is of *Messerschmidia argentea* (L.f.) Johnst. on the back shore of Krusadi island.

Soils are coral sand with pH of 7·2–8·2, organic matter varies from 0·50–2·72%; calcium carbonate from 50–80% and total soluble salts from 0·17–1·25% (Rao *et al* 1963b).

Sri Lanka

The sands occupy a large extent along the low lying coast of the dry zone from where they may be blown to the interior as in the Mannar Peninsula.

Beyond the limit reached by the waves, on recently deposited unstable sands is encountered low creeping vegetation formed of *Ipomoea pes-caprae, Canavalia rosea* DC., *Spinifex littoreus* which one also finds on the dunes nearest to the coast as also *Scaevola plumieri.* The common companion species are *Hydrophylax maritima* L. f., *Synostemon bacciforme* (L.) Webster, *Launaea sarmentosa, Zoysia matrella* Merr., *Cyperus pedunculatus.*

Towards the interior, the sand becomes less saline and a little more fertile; the outer most zone is composed of *Tephrosia purpurea, Hedyotis corymbosa* Wall., *Phyllanthus debilis* Hook. f., *Crotalaria retusa* L. Var. *maritima*; the next zone of bushes comprises *Scaevola taccada, Clerodendrum inerme, Pemphis acidula*, and *Morinda citrifolia.* Finally there remain some remnants of a littoral forest now replaced by coconut plantations like *Calophyllum inophyllum, Terminalia catappa* L., *Thespesia populnea, Barringtonia asiatica* Kurz, *Hibiscus tiliaceus* L., *Pongamia pinnata* (L.) Pierre and *Pandanus tectorius* Sol.

Salt marshes

These are located along the margins of numerous lagoons which mark the coast of Sri Lanka, particularly in the zone of a long dry season where evaporation notably increases their salinity. In relation to this factor and the variable frequency of these being reached by the tides are differentiated zones characterised by closely adopted species, usually in pure populations.

In the salt marshes bordering Mundel lake, Chapman (1947) recognised the following zones.

(i) Zone of *Cyperus haspan* L. and *Scirpus littoralis* Schrad.

(ii) Association of *Zoysia matrella* Merr. with occasionally some small trees like *Avicennia intermedia* Griff. and *Heritiera littoralis* Dryand.

(iii) Zone of *Suaeda monoica* Forsk. with some *Zoysia* and *Eleocharis setacea* (Retz.) R. Br.

(iv) Zone of *Suaeda monoica* Forsk. without any other species except occasional *Zoysia* and *Arthrocnemum.*

(v) A barren muddy zone with a fringe of blue-green algae here and there.

(vi) A zone of *Halophila ovata* Grand.

(vii) Mangrove zone dominated by *Avicennia intermedia.*

Salt pans

Species most resistant to salinity like *Salicornia* and *Suaeda* grow on their borders.

Halophilous pseudosteppes

Along the north-west coast of the island and to the south of the Jaffna lagoon occur black clayey soils with varied degrees of salinity; they bear a discontinuous grassy vegetation mixed with halophytes like *Tamarix gallica* L., *Suaeda* spp. as also some sparse trees like *Salvadora persica* L. and *Manilkara hexandra* (Roxb.) Dub.

There remain only a few patches in Sri Lanka. Most of these were cut down and drained for raising coconut plantations.

An excellent ecological study of a salt marsh of Sri Lanka located in the Gulf of Mannar is that of Pemadasa *et al* (1979). The authors recognised the following communities.

(a) *Suaeda*, (b) *Arthrocnemum* consociation, (c) Salt-marsh pastures, (d) Thorn Scrub, (e) Depressions.

9. The Utkal and Bengal Coast

An ecological study of the strand vegetation of the Utkal coast has been carried out by Rao and Mukherjee (1975). The strand flora situated on a raised sandy ridge from Balasore to Ganjam districts shows sufficient density towards inlands. The pioneers are *Canavalia maritima* (Aubl.) Thw., *Cyperus arenarius* Retz., *Fimbristylis junciformis* Kunth, *Launaea sarmentosa* and *Hydrophylax maritima* L. f. They are closely followed by *Euphorbia rosea* Retz., *Geniosporum tenuiflorum* (L.) Merr. and *Phyllanthus rotundifolius* Klein. The next set of strand builders are *Spinifex littoreus* (Burm. f.) Merr., *Borreria articularis, Ipomoea pes-caprae, Polycarpaea corymbosa, Portulaca tuberosa* Roxb. and *Perotis indica* (L.) O. Ktze. (figure 4).

The zonal pattern as stated above exhibits gradation in the substratum which is reflected in the variation of floristic composition.

Euphorbia rosea, Geniosporum tenuiflorum and *Hydrophylax maritima* encountered on the coastal sands of Utkal are not found further north of the Medinipur plain (Rao *et al* 1970); on the other hand, *Enicostema hyssopifolia* and *Aeluropus lagopoides* which are very common along the Indian coastline are not recorded from the Utkal coast (Rao 1971).

The fore-dunes in the vicinity of Digha have been described by Rao *et al* (1974). The newly recorded plants of Medinipur coast are *Aeluropus lagopoides, Cyperus esculentus* L., *Trianthema triquetra* Rottl., *Portulaca tuberosa* Roxb., *Gisekia pharnaceoides* L., *Rothia indica* (L.) Druce, *Spinifex littoreus* and *Syzygium ruscifolium* (Rao 1969).

10. Andaman and Nicobar islands

The strand vegetation occupies flat strips of limited length along the broken coast lines of these islands, varying from a few to several meters in width. In sheltered areas, dense strand vegetation is formed between the muddy flats and the interior. In some places shingles from weathered limestone cliffs or broken shells or corals constitute the beaches. The vegetation of the pioneer zone on the sandy relief is composed of *Ipomoea pes-caprae, Clitoria ternatea* L. and *Vigna lutea* Gray. The raised ridges in this area support *Scaevola taccada, Sophora tomentosa* L., *Erythrina indica* Lam. and

Figure 4. Percentage cover and zonal pattern in open pioneer-dune zone of Utkal coast near PURI.

Cynometra ramiflora L. The other notable trees are *Heritiera littoralis, Thespesia populnea, Ochrosia barbarila* Gmel., *Pongamia pinnata, Terminalia catapa* and *Calophyllum inophyllum.*

The coral strand relief, especially the raised consolidated ridges support *Cycas rumphii* Miq., *Scaevola taccada, Messerschmidia argentea* (L. f.) Johnst., *Caesalpinia crista* L. and *Manilkara littoralis* (Kurz) Dub. Along the coral sand the vegetation is composed of *Thuarea involuta* (G. Forst.) R. Br., *Cyperus pedunculatus, Scaevola taccada, Guettarda speciosa* L. and *Cycas rumphii.* The sandy areas in the Nicobar group of islands are composed of *Spinifex littoreus, Cyperus pedunculatus, Salomonia cantoniensis* Lour., *Euphorbia atoto* and *Wedelia biflora* DC.

11. Conclusions

Well developed stretches of the dry coastal ecosystems are rather rare in the sub-continent and only a few examples are available. One such stretch extends from Kozhikode to Kasargod along the Malabar coast where *Ipomoea-Canavalia* community forms a thick mantle on the sand as the area is not inhabited by the fishermen. Another example is the sandy bulge between Konarak and the mouth of the Chilka lake with the community of *Ipomoea-Hydrophylax* closely followed by *Spinifex-Geniosporum-Tephrosia.*

For the other areas of the sub-continent, the strand flora is considerably disturbed and altered by man. Under the multiple use management may be mentioned the Dwarka-Okha region of the north-west Kathiawar where gypsum is mined and sand is used for the manufacture of cement. Along the Malabar coast, shells are collected for various purposes and Thorium mineral is extracted.

Plantations of coconut almost throughout the entire coastline along with palmyra palm (*Borassus*), *Casuarina equisetifolia*, cashewnut (*Anacardium occidentale* L.) have considerably altered the original strand vegetation.

The non-calcareous beaches consist of fine grained sand and provide a good percolating field for the rain water. The good quality water stored at a depth of 5–10 m permits irrigation for agriculture and forest-nurseries.

Growing of crops like millets (*Pennisetum typhoides* (Burm.) Stapf et Hubb.), ground-nut (*Arachis hypogea* Willd.) with saline water irrigation in coastal tracts is still in an experimental stage at present.

The problem of shifting sand is noted in the Utkal coast, Coromandel coast, Malabar coast and in the Rameshwaram island. Active measures are needed to check the spread of the sand from invading the neighbouring cultivated land.

The growth-forms of the common strand plants are enumerated below after Rao (1971).

Growth-forms of common strand plants

(a) Mat-forming strand creepers.

Aeluropus lagopoides, Canavalia maritima, Canavalia cathartica, Indigofera aspalathoides, Ipomoea pes-caprae, Launaea sarmentosa, Paspalum vaginatum, Perotis indica, Sesuvium portulacastrum, Sporobolus virginicus, Trachys muricata (L.) Pers., *Zoysia matrella.*

(b) Diffusely branching prostrate/erect strand herbs and sedges

Allmania nodiflora Br., *Anotis carnosa* Hk. f., *Atriplex repens* Roth., *A. stocksii* Boiss., *Borreria articularis* (L. f.) F. N. Will., *B. stricta, Crotalaria nana, Enicostema hyssopifolium* (Willd.) Verdoon. *Euphorbia atoto, E. rosea, Geniosporum tenuiflorum* (L.) Merr., *Hydrophylax maritima, Polycarpaea corymbosa, P. spicata, Scaevola plumieri* (L.) Vahl.

(c) Strand climbers

Dalbergia spinosa Roxb., *Derris trifoliata, Flagellaria indica* L., *Ipomoea macrantha, Parsonsia helicandra.*

(d) Strand plants with perennating organs

Asparagus dumosus, Scilla hyacinthina (Roth.) Macbr., Urginea indica.

(e) Strand scrubs

Acrostichum aureum (Strand fern), *Clerodendrum inerme, Dimorphocalyx glabellus* Thw., *Halopyrum mucronatum* Stapf, *Myriostachya wightiana* (Nees) HK. f., *Scaevola taccada, Syzygium ruscifolium* (Willd.) Sant. & Wagh, *Tamarix articulata* Vahl.

(f) Strand trees

Acacia planifrons W. & A., *Ardisia littoralis, Capparis cartilaginea* Decne., *Calophyllum inophyllum, Euphorbia caducifolia, Hyphaene dichotoma, Messerschmidia argentea* (coral sand), *Morinda citrifolia, Pandanus tectorius, Pemphis acidula* (coral sand), *Premna serratifolia* L., *Salvadora persica* L.

The mat-forming strand creepers may be suitably used for the fixation of the moving sand and mobile sand-dunes. Rao (1977) has proposed a list of useful species for the conservation of the sandy soils of the different biogeographic regions of the sub-continent based on the ecological amplitude of the coastal species.

It is interesting to note that the salt pans are confined to the zones with a relatively long dry season and are absent in the regions where the season of heavy rains exceeds 4 months.

References

Arora R K and Aggarwal K R 1965 Observation on the vegetation of Malpe Coast and neighbouring islands. *J. Indian Bot. Soc.* **44** 314–325

Bagnouls F and Gaussen H 1953 Saison sèche et indice xérothermique. *Documents pour les cartes des productions végétales* to. III, Vol. 1, art. 8, 47 p. Toulouse.

Blasco F 1975 Les mangroves de l'Inde; *Inst. Fr. Pondichéry. Trav. Sec. Sci. Tech.* **14** 1–175

Blasco F 1977 Outlines of ecology, botany and forestry of the mangals of the Indian subcontinent. in Wet coastal ecosystems (ed.) V J Chapman (Oxford: Elsevier) pp. 241–260

Blasco F and Legris P 1973 Dry evergreen forest of Point Calimere and Marakkanam; *J. Bombay Nat. Hist. Soc.* **70** 279–294

Blatter E, Mc Cann C and Sabnis T S 1929 *The flora of the Indus Delta* (Madras)

Champion H G 1936 A preliminary survey of the forest types of India and Burma; *Indian For. Rec. (N.S.) Silv.* **1** 1–200

Chapman V S 1947 The application of aerial photography as exemplified by the natural vegetation of Ceylon: *Indian For.* **73** 287–314

Chapman V S (ed.) 1977 *Wet coastal ecosystems* (Oxford: Elsevier)

Eig A 1931 Les éléments et les groupes phytogeographiques auxiliaires dans la flore palestinienne. I Texte. II. Tableaux analytiques. *Repertorium specierum novarum regni vetabilis.* Beihefte Band LXIII, 201 p. Dahlem bei Berlin.

Marlange M and Meher-Homji V M 1965 A phytosociological study of the Pondicherry region. *J. Indian Bot. Soc.* **44** 167–182

Meher-Homji V M 1965 On the Sudano-Deccanian floral element; *J. Bombay Nat. Hist. Soc.* **62** 15–18

Meher-Homji V M 1970 Notes on some peculiar cases of phytogeographic distributions; *J. Bombay Nat. Hist. Soc.* **67** 398–413

Meher-Homji V M 1973 A phytosociological study of the *Albizia amara* Boiv. Community of India; *Phytocoenologia* **1** 114–129

Meher-Homji V M 1974 On the origin of the tropical dry evergreen forest of South India; *Int. J. Ecol. Environ. Sci.* **1** 19–39

Nayar M P 1959 The vegetation of Kanyakumari district; *Bull. bot. Surv. India* **1** 122–126

Pemadasa M A, Balasubramanian S, Wijewansa H G and Amarasinghe L 1979 The ecology of a salt marsh in Sri Lanka; *J. Ecol.* **67** 41–63

Rao T A 1969 The maritime strand flora along the Indian coast. A preliminary survey; *Proc. 56th Indian Sci. Congr.* **III** 432

Rao T A 1971 Distributional resume on the maritime strand flora of India; *Bull. Bot. Surv. India* **13** 192–202

Rao T A 1977 Management of coastal sandy biomes in India. Seminar on Afforestation. Inst. Public Health Engineers, Calcutta.

Rao T A 1978 On the coastal floristics and vegetation of the Malabar coast pertaining to Kerala State, India. The Madras Herbarium 125th Anniversary Souvenir Symp., p. 24.

Rao T A and Aggarwal K R 1964 Ecological studies of Saurashtra coast and neighbouring islands: III. Okhamandal point to Diu Coastal areas. Symp. Problems Indian Arid Zone, Jodhpur, pp. 31–42

Rao T A, Aggarwal K R and Mukherjee A K 1963a An ecological account of the vegetation of Rameswaram island; *Bull. Bot. Surv. India* **5** 301–325

Rao T A, Aggarwal K R and Mukherjee A K 1963b Ecological studies on the soil and vegetation of Krusadi group of islands in the Gulf of Mannar; *Bull. Bot. Surv. India* **5** 141–148

Rao T A and Korlahalli B C 1969 A note on the inflorescence of *Hyphaene indica* Becc; *J. Bombay Nat. Hist. Soc.* **66** 235–236

Rao T A and Mukherjee A K 1975 An ecological study on the strand vegetation of the Orissa coast; *Indian For.* **101** 692–701

Rao T A, Mukherjee A K and Banerjee L K 1970 Vascular plants of the coastal Midnapore district, W. Bengal; *Indian For.* **96** 668–677

Rao T A, Mukherjee A K and Banerjee L K 1974 A few unrecorded taxa for the flora of Kanyakumari shore (Cape Comorin), Tamil Nadu; *J. Bombay Nat. Hist. Soc.* **71** 346–349

Rao T A and Sastry A R K 1972 An ecological approach towards classification of coastal vegetation of India—I. Strand vegetation; *Indian For.* **98** 594–607

Rao T A and Sastry A R K 1974a An ecological approach towards classification of coastal vegetation of India. II. Estuarine border vegetation; *Indian For.* **100** 439–452

Rao T A and Sastry A R K 1974b An outline of the coastal vegetation of India; *Bull. Bot. Surv. India* **16** 101–115

Rao T A and Shanware P G 1967 Ecological studies of Saurashtra coast and neighbouring islands. VI. An approach to a classification of the Saurashtra coastland. A resume; *Bull. Bot. Surv. India* **9** 240–248

Rao T A, Shanware P G and Mukherjee A K 1974 Ecological studies on the coastal sand dunes and slacks in the vicinity of Digha, Midnapore district, W. Bengal; *Indian For.* **100** 101–107

Rolla S R 1963 *Hyphaene indica* Becc. along the west coast of India; *J. Bombay Nat. Hist. Soc.* **60** 761–763

Sastry A R K and Rao T A 1973 Studies on the flora and vegetation of coastal Andhra Pradesh, India; *Bull. Bot. Surv. India* **15** 92–107

Satyanarayan Y 1958 Ecological studies of Bombay coast line. I—Strand vegetation; *J. Biol. Sci.* **1** 53–65

Sebastine K M and Ellis J L 1967 A contribution to the vascular flora of Vedharanyam and Talignayar reserve forests, Tanjore district, Madras State; *Bull. Bot. Surv. India* **9** 190–200

Shah G L 1962 The vegetation along the seashores in Salsette Islands, Bombay; *Bull. Bot. Surv. India* **4** 239–249

Sprangers J T C M and Balasubramanian K 1978 A phytosociological analysis of the tropical dry semi-evergreen forest of Marakanam, South-Eastern India; *Trop. Ecol.* **19** 70–92

Srinivasan K S 1960 Aspects of vegetation of Church Island off Tuticorin port in South India; *J. Bombay Nat. Hist. Soc.* **57** 348–353

Stoddart D R and Fosberg F R 1972 South Indian sand cays. *Atoll Res. Bull.* **161** 1–16 Smithsonian Inst., Washington.

Sundararaj D D and Nagarajan M 1964 The flora of Hare and Church Islands off Tuticorin; *J. Bombay Nat. Hist. Soc.* **61** 587–602

Thomas K J 1962 A survey of the vegetation of Veli (Trivandrum) with special reference to ecological factors; *J. Indian Bot. Soc.* **41** 104–131

Viart M 1963 Contribution à l'étude de l'action de l'homme sur la végétation dans le sud de l'Inde. Thése d'Ingenieur Docteur, Faculté des Sciences, Toulouse, 259 p.

Werger M J A and Sprangers J T C M 1982 Comparison of floristic and structural classification of vegetation; *Vegetation* **50** 175–183

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 2 & 3, April & May 1985, pp. 525–537.
© Printed in India.

Floral differentiation and its modification

I USHA RAO and H Y MOHAN RAM
Department of Botany, University of Delhi, Delhi 110007, India

Abstract. Flower initiation is an important morphogenetic event. In this brief review the formative, ultrastructural, cytological and biochemical changes that occur in the transitional meristems in a few selected species have been discussed. In the evoked meristems, the number of plastids, mitochondria and ribosomes are usually higher. Further, an early shift of 4C nuclei to the 2C value, an increase in respiration and enhanced activities of dehydrogenase and phosphatase have been observed. The molecular events that ensue immediately after induction need further study.

The factors that regulate flower morphogenesis in vitro and reversal of excised flower buds to vegetative growth have been discussed. Reports on the modification of inflorescence development through the application of growth regulators have been analysed.

Keywords. Barren capitula; *Calendula*; *Chenopodium*; chlorflurenol; *Chrysanthemum*; floral differentiation; floral meristem; floral morphogenesis; floral organogenesis; gibberellic acid; inflorescence; long-day plants; reversal to vegetative growth; short-day plants; *Sinapis*; *Spinacea*; *Tagetes*; ultrastructure; virescent inflorescence; *Xanthium*.

1. Introduction

The transformation of a vegetative shoot apex into a reproductive one is a fundamental morphogenetic event. The causal factors of floral evocation have been studied in several angiosperms (Chailakhyan 1958; Lang 1965; Evans 1969), yet "how the reproductive apex, during its metabolism and growth gives rise to organs of characteristic size, position and symmetry and to the tissue pattern within" (Wardlaw 1965) is far from clear and has remained a subject of deep interest.

Flowering plants display wide variation in flowering response. The vegetative shoot apical meristem itself may be consumed in forming a flower or an inflorescence, or it may continue to form both vegetative structures and flowers.

The study of formative and histological changes in the vegetative and reproductive apices has been pursued by several investigators (Popham 1963; Cutter 1965; Lyndon 1978; Halperin 1978; Wareing 1978; Havelange 1980; Mohan Ram 1980; Bernier *et al* 1981). Precisely-timed measurements of morphological changes at the shoot apex during floral transition indicate that flower formation is usually accompanied or even preceded by several macroscopic changes which are often regarded as "symptoms" of flowering (see Bernier *et al* 1981) such as increased elongation of young internodes; precocious initiation of axillary buds, increased leaf growth rate, change in leaf shape, increased rate of initiation of leaves and primordia, enlargement and doming of the meristem, and increased phyllotaxis. Our knowledge of the cellular and sub-cellular events occurring in the meristems has been greatly enriched using plants such as *Sinapis alba* and *Spinacea oleracea* (long day plants) and *Xanthium strumarium* (short day plant) in which flowering can be induced by providing a single inductive cycle.

2. The floral meristem

2.1 *Ultrastructural changes*

Qualitative and quantitative ultrastructural changes in meristems of *Sinapis* and *Spinacea* have been studied by Havelange and co-workers (1974, 1980) and by Auderset and Greppin (1977). In *Sinapis*, the number of mitochondria per cell increases as early as 18 hr after start of the long day, and at the time of the initiation of the first flower primordia at 62 hr, this number is 2 to 3 times higher than at the start of induction. In *Spinacia* and *Xanthium* both plastids and mitochondria show an increase in their number. In the former the splitting of existing vacuoles has been noted 18 hr after induction. In *Spinacea* dumbbell-shaped or lobed plastids and mitochondria are frequently seen, suggesting that these are dividing. Frequent divisions of proplastids were also observed by Gifford and Stewart (1965) in the transforming meristems of *Chenopodium album*. Furthermore, *de novo* formation of flowers from explants containing multiple cell layers of tobacco is also characterized by an increase in the number of mitochondria (Tran Thanh Van and Trinh 1978).

The concentration of ribosomes is usually greater in the pre-floral meristems than in the vegetative meristems (Lance-Nougarède and Bronchart 1965; Lance-Nougarède 1967; Lin and Gifford 1976) and the complete structural organization of the nuclei is indicative of increased synthesis and export of ribosomal subunits. An increase in endoplasmic reticulum and dictyosome number and activity have been reported in *Sinapis*, *Spinacia*, *Xanthium* and *Chenopodium album* (Gifford and Stewart 1965). The nucleoli of evoked meristems are larger, less compact and more vacuolate, and within the nucleus an increased volume of dispersed chromatin is seen in *Sinapis*. Examination of Feulgen-stained preparations of transforming meristems in *Lolium* and cauliflower show chromatin decondensation (Knox and Evans 1976; Sadik and Ozbun 1967).

2.2 *Cytological changes in the floral meristem*

The most conspicuous event in floral transformation is the appearance of numerous mitotic figures. Using Feulgen cytophotometry to establish the distribution of the 2C and 4C cell populations Jacqmard and Miksche (1971) showed that in the vegetative (intermediate) meristems prior to LD induction, nuclei with 4C amount of DNA (G_2 nuclei) are more numerous than those with 2C DNA content (G_1 nuclei). In the transitional meristems, there is an early shift of 4C nuclei to the 2C value, and this leads to near-synchronization of the cell population at the 2C condition at the 30th hr, *i.e.*, at the completion of the first mitotic wave when 65 to 70 % of the total cell population is in G_1. The mean cell-doubling time in Petkus winter rye and lupin was estimated by Sunderland (1961) from counts of total cell numbers in apices and numbers of cells produced by them during a given time interval. Direct measurements of the cell-doubling time in each zone of transforming meristems can also be achieved by counting metaphase cell accumulation after colchicine treatment. Use of this technique by Corson (1969) in the day-neutral plant (DNP) *Datura* and by Bodson (1975) in the long-day plant (LPD) *Sinapis* reveals that cell-doubling times decrease in both the central and the peripheral zones of the meristem during transition to flowering. During floral transition a cell population in the meristem becomes transiently synchronized. In *Sinapis* there are two mitotic waves during the transition: the first one is clearly

associated with cell synchronization while the second, which occurs concurrently with the onset of flower initiation, is related to the increased rate of cell division (Bernier *et al* 1981). A single application of a low dose of a cytokinin made at midnight to the apical bud of a vegetative plant of *Sinapis alba* produces a mitotic wave similar in every respect to that found in the meristem of plants induced to flower by a single long day (Bernier *et al* 1974). It was, therefore, proposed that cytokinin may be one of the components of the complex floral stimulus in this species (Bernier *et al* 1977).

2.3 *Biochemical changes*

Transforming apices of *Iris* have higher rates of transpiration than their vegetative counterparts. Microrespirometric and histochemical studies by Thein (1957), Krekule and Teltscherova (1966) and Opatrná (1970) in the evoked meristems of both long- and short-day plants show that respiration and dehydrogenase activity increase during passage from the vegetative to the reproductive stage. A histochemical investigation by Jacqmard (1978) has revealed that succinic dehydrogenase activity increases significantly by the 22nd hr after the start of the inductive LD in the meristem of *Sinapis* and coincides with the increase in the size of the condriome found in the evoked meristem (Havelange *et al* 1974). These two events reflect an increase in cellular respiratory activity for the release of energy (Bodson 1977; Bodson *et al* 1977). A marked increase in the number of mitochondria accompanies these changes. Similar observations have been reported in the SDP *Xanthium* (Thein 1957; Havelange 1980). Localisation of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase by Gahan *et al* (1979) in the meristem sections of *Spinacia* indicates an augmentation in the activity of pentose phosphate pathway as a result of floral induction. There is a marked accumulation of starch in all parts of the apex during transition to flowering both in LDP's (Bernier 1971; Bodson 1977), SDP's (Gifford and Tepper 1962) and cold-requiring plants (Sadik and Ozbun 1967). The increase in RNA and protein content can be measured histochemically (Jacqmard *et al* 1972). The concentration of RNA in the apex of *Silene* increases by about 30% during induction as it does in many other plants (Miller and Lyndon 1976). New kinds of proteins specific to floral condition have not been detected (Stiles and Davies 1976). Just one or two new bands have been observed in electrophoretic analysis (Sawhney *et al* 1976). However, Barber and Steward (1968) had noted that the induction of flowering in *Tulipa* led to detectable qualitative protein changes "before the floral organs had developed".

An increase in acid phosphatase activity in evoked meristems of *Sinapis* was detected as early as 14 hr after the start of the inductive long day (Bernier *et al* 1981). This intensity was also found correlated with regions of rapid cell division in other systems (Wilson and Cutter 1955; Fosket and Miksche 1966; Shaykh and Roberts 1974). The increase in ribonuclease activity begins at 18 hr, a few hours after a rise in RNA synthesis in the 1·5 mm apical bud of the same species (Pryke and Bernier 1978). The results concerning acid phosphatase and ribonuclease show that catabolic reactions are also activated during evocation of flowering along with synthetic activities (Jacqmard 1978).

3. Flower morphogenesis in vitro

The technique of culturing young excised flower primordia introduced by LaRue (1942) affords a potentially useful tool for (a) controlling flower morphogenesis

(b) assessing the influence of growth regulators and nutrients on buds in the absence of intervening vegetative tissues; (c) testing the autonomy of the floral apex; and (d) performing a variety of surgical manipulations.

Johri and Ganapathy (1967), Wadhi (1967), Mohan Ram and Jaiswal (1975) and Konar and Kitchlue (1982) have reviewed the literature on flower morphogenesis in vitro. In general when excised immature flower buds are cultured they are able to produce viable pollen grains and ovules in very few instances. The work done by Tepfer *et al* (1963, 1966) on *Aquileqia* has demonstrated that the younger the flower bud at culture, the more elaborate are its growth requirements. Whereas Tepfer *et al* (1966) believed that IAA was necessary for carpel development, Bilderback (1972) failed to demonstrate its requirement. The role of gibberellic acid (GA_3) in the development of calyx, corolla and ovary in the excised immature flower buds in *Viscaria candida* was demonstrated by Blake (1966). In the cultured immature inflorescences of *Cyperus rotundus*, Mohan Ram and Batra (1970) noted that cytokinins promoted the origin and development of new flowers. Hicks and Sussex (1970) observed that initiation and early growth of organ primordia are independent of exogenously applied hormones, but that kinetin plays a key role in enhancing the eventual development of all the organ primordia.

The number of examples in which altered morphogenetic responses have been noted in the cultured immature flower buds are numerous. Callusing, production of roots, shoots or entire plantlets through differentiation of embryoids have been reported by Konar and Nataraja (1964), Konar and Konar (1966), Mohan Ram and Wadhi (1966), and Ganapathy (1969). Greyson and Raman (1975) studied *Nigella damascena* using genotypically dissimilar 'single' and 'double' flowers, the 'double' being inherited as a single gene recessive. Floral apices when cultured on Murashige and Skoog's (MS) medium supplemented with kinetin (Kn) supported stamen and carpel initiation in both the genotypes, whereas 'single' could do so on MS medium alone. In the medium minus (Kn), GA_3 was essential for organ initiation in the 'double', but it totally inhibited stamen initiation in the 'singles'. Raman and Greyson (1978) further observed that GA_3 inhibited the initiation and growth of stamens and nectaries in 'singles'. The use of IAA or Kn in any combination failed to overcome the inhibitory action of GA_3 on 'singles'. These authors have also succeeded in obtaining graft unions between bisected floral meristems of the two forms (Raman and Greyson 1977).

A rather difficult problem to tackle is the spatial relationship in regard to the origin of organ primordia in the floral apex. At what point of ontogeny does the specific pattern of organ primordia become blocked in the nascent meristem? If it is conceived that the floral apex is a mosaic of irrevocably determined sites of different organs, the removal of a particular part of the flower should cause an irreparable loss of the part (Heslop-Harrison 1972). It could also be presumed alternatively, that the sequences of organ initiation is not predetermined and the terminal tissue of a floral meristem is capable of replacing the lost parts (Mohan Ram and Jaiswal 1975). Experiments dealing with bisected and cultured immature flower buds have shown that floral organs are able to regenerate even at the cut surfaces (Jensen 1971). Hicks (1972) studied the fate of bisected stamen primordia at different developing stages while they were still attached to the floral apex. Remarkably enough, he observed that two stamens, each containing four anther lobes developed from a bisected young stamen primordium. However, such regeneration was not possible with older stamens. Whereas GA_3 causes the production of stamen in the stamenless-2

refloral tomato mutants (Sawhney and Greyson 1973), the work of Hicks and Sand 1977) has established that undifferentiated stamen primordia of a male sterile tobacco ybrid in vitro are capable of relatively autonomous development and are not nfluenced by the hormone.

.1 *Stability or instability of the floral state in vitro*

nflorescence segments of day-neutral tobacco plants (DNP's) regenerate flower rimordia in vitro, whereas inflorescence segments of daylength-sensitive tobacco arieties produce only vegetative buds. This differential behaviour was explained by hailakhyan *et al* (1975) in the following manner: in DNP's all components of the floral ormone complex are synthesized in all plant parts independently of daylength and are resent in explants of inflorescence axes which can thus initiate flowers in vitro. On the ontrary, in daylength-sensitive plants the hormonal complex is believed to be formed nly in leaves that are exposed to the appropriate photoperiod. In tissue explants xcised from the inflorescence of these plants, the hormonal complex is rapidly xhausted due to absence of leaf tissues and only vegetative buds are regenerated. As redicted by Chailakhyan, inflorescence fragments of the SDP *Streptocarpus nobilis* roduce only vegetative buds in vitro even when cultivated in inductive SD, whereas iscs collected from leaves regenerate flower buds in SD, but not in LD (Rossini 1970).

. Reversion of reproductive apices to vegetative growth

n nature vegetative structures may arise spontaneously within positions of flowers in any species. These malformations were termed "teratisms" by morphologists. With he discovery that the passage of plants to the reproductive condition is frequently overned by certain environmental parameters, physiologists succeeded in producing uch "chimeric" flowers (or inflorescences) (Von Witsch 1965). Usually such structures ppear in some photoperiodic and cold-requiring species given marginal, interrupted, r otherwise perturbed induction. Occurrence of a graded series or vegetative-eproductive interphases suggests that apices that have reached the realization reproduction) stage can revert to a more or less typical vegetative condition (Von Vitsch 1965).

When a transitional meristem of *Petasites* presumably at the early prefloral stage is isected, punctured at the centre, or isolated from adjacent subapical tissues by vertical ncisions, the small, simple bracts develop into large, petiolate and laminate leaves, and o florets are initiated. At later stages of capitulum development similar surgical reatments no longer impede flower formation (Wardlaw 1963).

.1 *Reversal of floral explants to vegetative condition*

he development of leafy shoots from excised flower buds is extremely rare. LaRue 942) observed the occurrence of leafy shoots in cultured floral buds in two species *Kalanchoe globulifera* and *Nemesis strumosa*) out of 92 spp of angiosperms. According o him the shoot buds had been formed prior to culture. Mohan Ram and Wadhi (1966, 968) reported reversion from floral to vegetative condition in *Kalanchoe pinnata*. hese authors cultured flower buds of two early stages of development on modified

White's medium with supplements. The buds failed to attain full development and the primordia of floral organs lost their ability for normal morphogenesis under culture conditions. The authors attributed the phenomenon of reversion to vegetative growth in *Kalanchoe* to early excision, which apparently hinders the normal sequence of floral morphogenesis.

4.2 *Inflorescence reversions*

Inflorescence reversions, *i.e.*, inflorescences that have acquired in their last-formed portions several or all characters of vegetative shoots, are known to occur spontaneously in plants such as *Ananas* and *Lavandula stoechas*. Experimental reversion of the inflorescence can be brought about in the Labiatae such as *Perilla* and *Salvia*, by transfer of these plants to unfavourable daylength regimes, but only if induction is suboptimal (see Bernier *et al* 1981). Reversions also commonly occur in *Nicotiana glutinosa* and *Hyoscyamus* (Diomaiuto-Bonnand 1969; Seidlova and Stichova 1965), brussel sprouts (Stokes and Verkerk 1951), *Sinapis* (Bagnard *et al* 1972; Bagnard 1978) and *Cheiranthus cheirii* (Diomaiuto-Bonnand 1972). Inflorescence proliferation may also occur in some grasses as a result of incomplete induction (Evans 1964). These morphological anomalies can be traced to spikelet proliferation, *i.e.* spikelets with an apical meristem continuing growth and producing a leafy shoot, and are consequently due to failure of determination in spikelet primordia which are uncommitted at initiation (Evans 1964).

5. Special cases

An ontogenetic study of reproductive structures in cauliflower (*Brassica oleracea* var. *botrytis*) by Sadik and Ozbun (1967) has revealed that the apical vegetative meristem is first transformed into a typical prefloral meristem, similar to the prefloral meristem of *Sinapis* or other crucifers, with precocious initiation of axillary meristems. In cauliflower, however, the prefloral stage is long-lasting, and the process of branching is enormously amplified. With time, second-order axillary meristems are initiated around meristems of first order branches, and later, third- and higher-order axillary meristems are initiated. This process continues until a very large number of meristems and branches accumulate and form the "curd". Although these meristems do not differentiate into flower primordia, the curd is obviously no longer a vegetative structure; rather it is an amplified prefloral inflorescence axis (Sadik and Ozbun 1967).

In some species such as *Phytolacca decandra* and tomato with sympodial growth, inflorescences occupy neither terminal nor strictly axillary positions. In these plants, the shoot apical meristem is normally transformed into a reproductive meristem after a certain period of vegetative functioning. Concomitantly with this transformation, vegetative growth is resumed by the development of a nearby vegetative meristem which assumes terminal position: now the initial reproductive meristem appears in a lateral position. After formation of a number of leaves, the new terminal meristem flowers and is in turn displaced laterally by a new vegetative meristem soon (Lance-Nougaréde and Rondet 1957).

In grapevine (*Vitis vinifera*) inflorescence primordia also arise in specialized axillary buds, the so-called "bourgeons latens" (Srinivasan and Mullins 1979). The first step in

the formation of inflorescences in these buds is the initiation of an extra-lateral meristem called "anlage" by the apical meristem. The anlage rapidly produces a bract on its abaxial side and then another lateral meristem on the opposite side. This adaxial meristem or "inner arm" becomes the main body of the inflorescence primordium if conditions are conducive to flowering, and the "outer arm" (adjacent to the bract) develops into the lower branch of the inflorescence primordium. These specialized buds of grapevine are apparently irreversibly evoked and committed to determinate growth early in their ontogeny. If conditions are not favourable to flowering, the two arms do not undergo branching, and the anlage then develops into tendrils which, like thorns are typically determinate structures (Srinivasan and Mullins 1979).

6. Floral organogenesis

Various contradictory conceptual models have been proposed for interpreting the flower (see Sattler 1965; Meeuse 1966; Eyde 1975). One such model explains the flower as a modified monaxial shoot (Eames 1961; Von Bartalanffy 1965; Cronquist 1968; Takhtajan 1969). According to another model, the basic unit of flower is the gonophyll (Melville 1962–1963). Meeuse (1966) uses the model of an anthocorm and gonoclad for the interpretation of flowers while Croizat (1960, 1962, 1964) thinks of a flower in a less rigid way as an axis with scales, some or all of which are sexualized. He distinguishes two types of flowers—the panstrobilar and the circumnucellar.

Present day developmental morphology lays emphasis on causal interrelations. This aspect of enquiry necessarily leads to the study of lower levels of organization such as cells, organelles, and macromolecules. As a result, cell biology and molecular biology have become dominant fields of enquiry, relegating comparative studies of organs, whole organisms, and their development to the background. Sattler (1973) emphasizes how much is yet to be learnt from comparative organogenesis of an almost ubiquitous organ-system such as the flower.

Two techniques have been used to study floral organogenesis. One is the familiar microtechnique (Johansen 1940; Feder and O'Brien 1968) which involves serial sectioning of floral buds and their subsequent reconstruction. The second is the dissection of floral buds and their photography under alcohol, using close-up lenses (Sattler 1968). Whereas sections reveal cytological and histological details, their utility in obtaining a geometrical picture is limited. The second technique offers a direct three-dimensional view of the floral buds, their primordial appendages and protodermal cells, but no internal tissue details. The limits of observation have been extended through the use of SEM, in which the minutest details of formative changes can be traced. The scanning pictures permit exact measurements of phyllotactic parameters in the young flower (Lyndon 1978).

7. Development of the capitulum and its modification

The capitulum of the Compositae has been an object of biological curiosity because of the compaction of florets on the axis with a centripetal diversification. There is an enormous increase in the area that the shoot apex undergoes in forming a capitulum [nearly 400-fold in *Chrysanthemum* (Schwabe 1959)] and in the differentiation of a multitude of floret growth centres in the place of a single vegetative meristem. The

capitulum of sunflower, which simulates a single flower, is probably the largest inflorescence (Heiser in 1976 recorded a 'two and one-half feet' sunflower) in the family and also the most complicated in phyllotaxis, with innumerable intersecting spirals of florets (Leppik 1977). Details of the topographical and floral organogenetic changes occurring during the transformation of a vegetative meristem into a capitulum have been studied by Popham and Chan (1952) in *Chrysanthemum*. In *Calendula officinalis*, chlorflurenol—a morphactin—suppresses the development of involucral bracts to various degrees, prevents full expansion of the thalamus and either inhibits the initiation of the florets or interferes with the completion of development of those florets which are already differentiated. Total elimination of only disc florets, or ray florets or a reduction in the number and size of the florets, leaving a vacant surface of the thalamus, either in the centre or along the periphery or between the florets have also been observed in *Calendula* and *Tagetes* (Mohan Ram and Mehta 1973; Mehta 1976; Chandra 1982). Various degrees of fusion of florets have been observed.

Chlorflurenol drastically retards the size of the capitula, by inhibiting the mitotic activity of the tunica, pith rib meristem and intercalary regions, and poor differentiation of the procambium in *Calendula officinalis*. Meristematic activity is also absent in barren capitula of *Tagetes*.

Bennink (1973) showed that under long days, all the initiated floral buds develop into "crown buds" each consisting of a capitulum and bracts without flower primordia, reminiscent of the barren capitula induced by chlorflurenol in *Calendula*. When a solution of benzylandenine (BA) was injected into the plants under long days, it induced the formation of normal flowers (Bennink 1973).

The barren capitula in both *Calendula* and *Tagetes* are rich in starch which may be a consequence of growth inhibition. However, cell division, cell expansion and morphogenetic activity inherent in a meristem are not irreversibly inhibited by chlorflurenol. When the barren capitula are cultured on a medium supplemented with IAA + GA_3 + Kn, mitotic activity resumed in the tunica, pith rib and intercalary regions. Structures resembling floret primordia and vegetative shoot buds were also initiated on the surface of the barren capitula in *Calendula* (Mehta and Mohan Ram 1978). In *Tagetes*, the barren capitula cultured on B_5 medium + BAP (10^{-5} M) callused from the cut end as well as from the surface and subsequently gave rise to shoot buds which could be excised and rooted to obtain flowering plants. Occasionally structures intermediate between shoot and florets appeared directly from the surface of the cultured capitulum and via a callus in *Tagetes* (Mohan Ram and Mehta 1982).

Regeneration of plantlets from fully developed capitula of *Gerbera jamesonii* has been reported by Pierik *et al* (1973, 1975). In these cultures, shoot buds developed from the base of the involucral bracts or from the receptacle. Development of adventitious shoots from the receptacle, remnants of disc florets, axils of disc florets or involucral bracts was reported in *Chrysanthemum cinerariaefolium* (Roest and Bokelmann 1973).

According to the classical concept, the flower is a metamorphosed vegetative shoot of limited growth in which the floral leaves arise in close succession either in spirals or cycles, the meristem being generally used up in their formation. Even botanists of the previous century had observed that in some varieties of roses and pears a secondary flower grew out from the centre of the first flower. This indicated that under certain conditions the floral meristem was not consumed but continued to grow. Occasional occurrence of secondary inflorescences has also been noted in certain members of the Compositae and the Dipsacaceae. Schwabe (1951) reported several teratological

features in *Chrysanthemum* such as formation of bracts on the receptacle as well as secondary inflorescences and petaloid stamens in the inflorescences of plants exposed to extended periods in long days. Bose and Nitsch (1970) reported that gibberellic acid causes this phenomenon in *Calendula officinalis*. Mohan Ram and Mehta (1978) also showed that GA_3 stimulated the formation of secondary and even tertiary inflorescences. Thus gibberellins are involved in the transformation of a floret primordium with a committed and limited developmental plan into a primordium of a secondary inflorescence with a greater morphogenetic potentiality. The peripheral florets, particularly the ray florets, appear to be labile in this respect.

In *Calendula officinalis*, a curious phenomenon observed under Delhi conditions late in the flowering season (April–May onwards, marked by lengthening of the photoperiod from 10–12 hr and a sudden rise in the mean day temperature (from about 20°C to 35 ± 5°C) was the replacement of the colourful capitula by green witches' broom-like structures termed "virescent inflorescences". This is manifested by the development of vegetative shoots from the base of the ovary in place of the ovule in the florets. These penetrate the ovary wall and emerge as leafy shoots (Rao and Mohan Ram 1984). It is also believed that mycoplasma-like organisms (MLO's) are able to cause virescent capitula in the Compositae (S Misra and A Varma, personal communication).

There are numerous instances in the literature of the development of bulbils from flower buds (*Agave*, *Yucca*, *Nymphaea*, *Allium*, etc.) although the causal factors are not established. Dúpuy (1963) and Guédés and Dúpuy (1979) have studied in detail the teratological modifications in the flowers of several genera.

8. Conclusions

Although earlier studies relating to reproductive development were mainly restricted to observational changes at the morphological or histological level, application of recent techniques of histochemistry, biochemistry, electron microscopy, and tissue and organ culture have enabled a better understanding of the causal aspects of floral development. The molecular events of floral evocation are still not fully known. The role of new messenger RNA's, and the changes in protein composition during floral transition of the meristem and whether the control of these changes is at the transcriptional level, the translational level, or beyond are not yet understood. Experimental modification of differentiation has demonstrated the role of environmental factors and growth regulators in determining or reversing the events of floral developments.

Acknowledgements

The authors thank Dr I V Ramanuja Rao, Miss K Prema, Mrs Vibha Narang and Messrs M Narasimham and Amar Singh for their help in the preparation of the manuscript.

References

Auderset G and Greppin H 1977 Effect de l'induction florale sur l'evolution ultrastructurale de l'apex caulinaire de *Spinacia oleracea* Nobel; *Protoplasma* **91** 281–301

Bagnard C 1978 *Etude experimentale de la reversion d'inflorescence chez Sinapis alba* Ph.D. Thesis, University of Dijon, France

Bagnard C, Bernier G and Arnal C 1972 *Physiol. vég.* **10** 237–250

Barber S T and Steward F C 1968 The proteins of *Tulipa* and their relation to morphogenesis; *Dev. Biol.* **17** 326–349

Bennink G J H 1973 Flower development in *Chrysanthemum* under long-day conditions by injections with the cytokinin benzyladenine; in *Plant growth substances* (Tokyo: Hirokawa) 974–979

Bernier G 1971 Structure and metabolic changes in the shoot apex in transition to flowering; *Can. J. Bot.* **49** 803–819

Bernier G, Kinet J M and Sachs R M 1981 *The physiology of flowering* (Roca Raton, Florida: CRC Press, Inc.) Vol 2

Bernier G, Kinet J M, Bodson M, Rouma Y and Jacqmard A 1974 Experimental studies on the mitotic activity of the shoot apical meristem and its relation to floral evocation and morphogenesis in *Sinapis alba*; *Bot. Gaz.* **135** 345–352

Bernier G, Kinet J M, Jacqmard A, Havelange A and Bodson M 1977 Cytokinin as a possible component of the floral stimulus in *Sinapis alba*; *Plant Physiol.* **60** 282–285

Bilderback E D 1972 The effects of hormones upon the development of excised floral buds of *Aquilegia*; *Am. J. Bot.* **59** 525–529

Blake J 1966 Flower apices cultured in vitro; *Nature (London)* **211** 990–991

Bodson M 1975 Variation in the rate of cell division in the apical meristem of *Sinapis alba* during transition to flowering; *Ann. Bot.* **39** 547–554

Bodson M 1977 Changes in the carbohydrate content of the leaf and apical bud of *Sinapis* during transition to flowering; *Planta* **135** 19–23

Bodson M, King R W, Evans L T and Bernier G 1977 The role of photosynthesis in flowering of the long-day plant *Sinapis alba*; *Aust. J. Plant Physiol.* **4** 467–478

Bose T K and Nitsch J P 1970 Induction of secondary inflorescence in *Calendula* by gibberellic acid; *Naturwissenschaften* **57** 254–255

Chailakhyan M Kh 1958 Hormonal factors of plant flowering; *Fiziol. Rast.* **4** 541–560

Chailakhyan M Kh, Aksenova N P, Konstantinova T N and Bavrina T V 1975 The callus model of plant flowering; *Proc. Roy. Soc. London* **190** 333–340

Chandra G 1982 *Morphogenesis and keeping quality of capitula in marigold*, Ph.D. Thesis, University of Delhi, India

Corson G E Jr 1969 Cell division studies in the shoot apex of *Datura stramonium* during transition to flowering; *Am. J. Bot.* **56** 1127–1134

Croizat L 1960 *Principia Bot.* 2 vols, Codicate

Croizat L 1962 *Space. Time. Form: The biological synthesis*; Caracas

Croizat L 1964 Thoughts on high systematics, phylogeny and floral morphogeny, with a note on the origin of the angiosperms; *Candollea* **19** 17–96

Cronquist A 1968 *The evolution and classification of flowering plants*; (Boston: Houghton Mifflin Co.)

Cutter E G 1965 Recent experimental studies of the shoot morphogenesis; *Bot. Rev.* **31** 7–113

Diomaiuto-Bonnand J 1969 *C.R. Hébd. Acad. Sci.* **268** 51

Diomaiuto-Bonnand J 1972 *C.R. Hébd. Acad. Sci.* **274** 372

Dúpuy P 1963 *Contribution a l'etude de quelques problems de morphologie et de teratologie chez les angiosperms* Ph.D. Thesis Poitiers, France

Eames A J 1961 *Morphology of the angiosperms* (New York: McGraw-Hill)

Evans L T 1964 *Grasses and grasslands* (ed.) C Barnard (London: Macmillan) 126

Evans L T 1969 *The induction of flowering: Some case histories* (Ithaca, New York: Cornell Univ)

Eyde R H 1975 The foliar theory of the flower; *Am. Sci.* **63** 430–437

Feder N and O'Brien T P 1968 Plant microtechnique; Some principles and new methods; *Am. J. Bot.* **55** 123–142

Fosket D E and Miksche J P 1966 Histochemical study of the seedling shoot apical meristem of *Pinus lambertiana*; *Am. J. Bot.* **53** 694–702

Gahan P B, Auderset G and Greppin H 1979 Pentose phosphate pathway activity during floral induction in *Spinacea oleracea* var. Nobel; *Ann. Bot.* **44** 121–124

Ganapathy P S 1969 Floral morphogenesis and flowering in aseptic cultures of *Browallia demissa* L.; *Biol. Plant* **11** 165–174

Gifford E M and Tepper H B 1962 Ontogenetic and histochemical changes in the vegetative shoot tip of *Chenopodium album*; *Am. J. Bot.* **49** 902–911

Gifford E M Jr and Stewart K D 1965 Ultrastructure of vegetative and reproductive apices of *Chenopodium album*; *Science* **149** 75–77

Greyson R I and Raman K 1975 Differential sensitivity of "double" and "single" flowers of *Nigella damascena* (Ranunculaceae) to emasculation and to GA_3; *Am. J. Bot.* **62** 531–536

Guédés M and Dupúy P 1979 *Teratological modifications and the meaning of flower parts* (India: Today and Tomorrow Publishers)

Halperin W 1978 Organogenesis at the shoot apex; *Ann. Rev. Plant. Physiol.* **29** 239–262

Havelange A 1980 The quantitative ultrastructure of the meristematic cells of *Xanthium strumarium* during the transition to flowering; *Am. J. Bot.* **67** 1171–1178

Havelange A, Bernier G and Jacqmard A 1974 Descriptive and quantitative study of ultrastructural changes in the apical meristem of mustard in transition to flowering. II. The cytoplasm, mitochondria and proplastids; *J. Cell Sci.* **16** 421–432

Heiser C S Jr 1976 *The sunflower* (Oklahoma Univ: Norman)

Heslop-Harrison J 1972 Sexuality of angiosperms; in *Plant physiology; A treatise* (ed.) F C Steward (New York: Academic Press) 133–289

Hicks G S 1972 Development in vitro of surgically halved stamen primordia of tobacco; *Can. J. Bot.* **50** 2396–2400

Hicks G S and Sussex I M 1970 Development in vitro of excised flower primordia of *Nicotiana tabacum*; *Can. J. Bot.* **48** 133–139

Hicks G S and Sand A A 1977 In vitro culture of the stamen primordia from male sterile tobacco; *Plant Sci. Lett.* **10** 257–263

Jacqmard A 1978 Histochemical localization of enzyme activity during floral evocation in the shoot apical meristem of *Sinapis alba; Protoplasma* **94** 315–324

Jacqmard A and Miksche J P 1971 Cell population and quantitative changes of DNA in the shoot apex of *Sinapis alba* during floral induction; *Bot. Gaz.* **132** 364–371

Jacqmard A, Miksche J P and Bernier G 1972 Quantitative study of nucleic acids and proteins in the shoot apex of *Sinapis alba* during transition from the vegetative to the reproductive condition; *Am. J. Bot.* **59** 714–721

Jensen L C W 1971 Experimental bisection of *Aquilegia* floral buds cultured in vitro 1. The effect on growth, primordia initiation, and apical regeneration; *Can. J. Bot.* **49** 487–493

Johansen D A 1940 *Plant microtechnique* (McGraw-Hill: London)

Johri B M and Ganapathy P S 1967 Floral differentiation and morphogenesis in vitro; *J. Indian Bot. Soc.* **46** 374–388

Konar R N and Kitchlue S 1982 Flower culture; in *Experimental embryology of vascular plants* (ed.) B M Johri (Berlin: Springer-Verlag) 53–78

Konar R N and Konar A 1966 Plantlet and flower formation in callus cultures from *Phlox drummondii* Hook; *Phytomorphology* **16** 379–382

Konar R N and Nataraja K 1964 In vitro control of floral morphogenesis in *Ranunculus sceleratus* L.; *Phytomorphology* **14** 558–563

Knox R B and Evans L T 1966 Inflorescence initiation in *Lolium temulentum* L. VIII. Histochemical changes at the shoot apex during induction; *Aust. J. Biol. Sci.* **19** 233–245

KreKule J and Teltscherova L 1966 Mikrorespirometrische Bestimmung der Atmungsintensität and Vegetationskegeln von Weizenpflanzen beim Übergang ven der vegetativen zur generation Entiwicklungsphase; *Biol. Plant* **8** 299–304

La Rue C D 1942 The rooting of flowers in sterile culture; *Bull. Torrey Bot. Club* **69** 332–341

Lance-Nougaréde A 1967 Experimental cytology of shoot apical cells during vegetative growth and flowering; *Int. Rev. Cytol.* **21** 203–351

Lance-Nougaréde A and Bronchart R 1965 Metabolisme des acides nucleiques dans le meristeme apical du *Perilla nankinensis* an cours des diverses phases du development; *C.R. Hébd. Acad. Sci. (Paris)* **245** 712–715

Lance-Nougaréde A and Rondet P 1957 Evolution der meristeme apical de *Beta vulgaris* L. de la germination a' l' inflorescence; *C.R. Hébd Acad. Sci. (Paris)* **245** 712–715

Lang A 1965 Physiology of flower formation; in *Encyclopaedia of plant physiology* (ed.) W Ruhland (Berlin: Springer-Verlag) 1380–1536

Leppik E E 1977 The evolution of capitulum types of the Compositae in the light of insect-flower interaction; in *The biology and chemistry of the Compositae* (eds) V H Heywood, J B Harborne and B L Turner (London: Academic Press) 61–90

Lin J and Gifford E M Jr 1976 The distribution of ribosomes in the vegetative and floral apices of *Adonis aestivalis*; *Can. J. Bot.* **54** 2478–2483

Lyndon R F 1978 Flower development in *Silene*: morphology and sequence of initiation of primordia; *Ann. Bot.* **42** 1343–1348

Meeuse A D J 1966 *Fundamentals of phytomorphology* (New York: Ronald Press)

Mehta U 1976 *Development of scotch marigold (Calendula officinalis L.) and its modification by some plant growth regulators* Ph.D. Thesis, University of Delhi, India

Mehta U and Mohan Ram H Y 1978 Modification of the capitulum in *Calendula officinalis* by chlorflurenol; *Phytomorphology* **28** 351–369

Melville R 1962–1963 A new theory of the angiosperm flower. I The gynoecium. II. The androecium; *Kew Bull. R. Bot. Gdn.* **16** 1–50 **17** 1–63

Miller M B and Lyndon R F 1976 Role of growth and cell division in the shoot apex of *Silene* during the transition to flowering; *J. exp. Bot.* **27** 1142–1153

Mohan Ram H Y 1980 *The biology of flower: Some aspects*. Presidential address 67th session of the Indian Science Congress Association: Calcutta 1–30

Mohan Ram H Y and Batra M 1970 Stimulation of flower formation by cytokinins of *Cyperus rotundus*; *Phytomorphology* **20** 22–29

Mohan Ram H Y and Jaiswal V S 1975 Some aspects of flower morphogenesis in vitro; in *Form, structure and function in plants* (eds) H Y Mohan Ram, J J Shah and C K Shah (India: Sarita Prakashan) 236–241

Mohan Ram H Y and Mehta G 1982 Regeneration of plantlets from cultured morphactin-induced barren capitula of African marigold (*Tagetes erecta* L.); *Plant. Sci. Lett.* **26** 227–232

Mohan Ram H Y and Mehta Usha 1973 Retardation of inflorescence development in *Calendula officinalis* by a morphactin and its application; *Biol. Plant.* **15** 152–154

Mohan Ram H Y and Mehta Usha 1978 Origin and development of secondary capitula in *Calendula officinalis* L. in response to gibberellic acid; *J. Exp. Bot.* **29** 653–662

Mohan Ram H Y and Wadhi M 1966 Reversion of floral buds of *Kalanchoe pinnata* to vegetative state in culture; *Naturwissenschaften* **53** 381–388

Mohan Ram H Y and Wadhi M 1968 Morphogenetic potentialities of flower buds of *Kalanchoe pinnata* grown in vitro; *Ann. Bot.* **32** 825–832

Opatrná J 1970 Histochemical localization of some dehydrogenases in shoot meristem of wheat of different stages of development; in *Cellular and Molecular Aspects of Floral Induction* (ed.) G Bernier (Longman: London) 80–90

Pierik R L M, Steegman H H M and Marelis J J 1973 *Gerbera* plantlets from in vitro cultivated capitulum explants; *Sci. Hortic.* **1** 117–119

Pierik R L M, Jansen J L M, Maasdam A and Binnendjilk C M 1975 Optimization of *Gerbera* plantlets production from excised capitulum explants; *Sci. Hortic.* **3** 351–357

Popham R A 1963 Developmental studies of flowering; in *Meristems and differentiation* Symp. Vol. Brookhaven National Laboratory (New York: Upton) 138–156

Popham R A and Chan A P 1952 Origin and development of the receptacle of *Chrysanthemum morifolium*; *Am. J. Bot.* **39** 329–339

Pryke J A and Bernier G 1978 RNA synthesis in the apex of *Sinapis alba* in transition to flowering; *J. Exp. Bot.* **29** 953–961

Raman R and Greyson R I 1977 Graft unions between floral half-meristems of differing genotypes of *Nigella damascena*; *Plant Sci. Lett.* **8** 367–373

Raman R and Greyson R I 1978 Further observations on the differential sensitivities to plant growth regulators by culture of "single" and "double" flower buds of *Nigella damascena* L. (Ranunculaceae); *Am. J. Bot.* **65** 180–191

Rao I U and Mohan Ram H Y 1984 Development of virescent capitula and conversion of florets to vegetative shoots in *Calendula officinalis*; *Phytomorphology* **34** 243–246

Roest S and Bokelmann G S 1973 Vegetative propagation of *Chrysanthemum cinerariaefolium* in vitro; *Sci. Hortic.* **1** 120–122

Rossini L M E 1970 in *Cellular and molecular aspects of floral induction* (ed.) G Bernier (London: Longman) 383

Sadik S and Ozbun J L 1967 Histochemical changes in the shoot tip of cauliflower during floral induction; *Can. J. Bot.* **45** 955–959

Sattler R 1965 Flower; in *McGraw-Hill year book of science and technology* (New York: McGraw-Hill)

Sattler R 1968 A technique for the study of floral development; *Can. J. Bot.* **46** 720–722

Sattler R 1973 *Organogenesis of flowers* (A photographic text atlas); (Canada: Univ. Toronto)

Sawhney V K and Greyson R I 1973 Morphogenesis of the stamenless 2 mutant in tomato. II. Modifications of sex organs in the mutant and normal flowers by plant hormones; *Can. J. Bot.* **51** 2473–2479

Sawhney S, Sawhney N and Nanda K K 1976 Gel electrophoresis studies of proteins in photo-induced and vegetative plants of *Impatiens balsamina*; *Plant Cell Physiol.* **17** 751–755

Schwabe W W 1951 Factors controlling flowering in the *Chrysanthemum*. II. Day-length effects on the further development of inflorescence buds and their experimental reversal and modification; *J. Exp. Bot.* **11** 223–237

Seidlova F and Stichova J J 1965 The anatomy of the shoot apex of *Hyoscyamus niger* L. reversed from the generative to the vegetative state; *Biol. Plant* **7** 376–379

Shaykh M M and Roberts L W 1974 A histochemical study of phosphatases in root apical meristems; *Ann. Bot.* **38** 165–174

Srinivasan C and Mullins M G 1979 Reproductive anatomy of the grape vine (*Vitis vinifera* L.): origin and development of the anlage and its derivatives; *Ann. Bot.* **38** 1079–1084

Stiles J C Jr and Davies P J 1976 Qualitative analysis by isoelectric focussing of the protein content of *Pharbitis nil* apices and cotyledons during floral induction; *Plant Cell Physiol.* **17** 855–857

Stokes P and Verkerk K 1951 *Meded. Landbouwhogesch Wageningen* **50** 141

Sunderland N 1961 Cell division and expansion in the growth of shoot apex; *J. Exp. Bot.* **12** 446–457

Takhtajan A 1969 *Flowering plants: Origin and dispersal*; (Edinburgh: Oliver and Boyd)

Tepfer S S, Greyson R I, Craig W R and Hindman J L 1963 In vitro culture of floral buds of *Aquilegia*; *Am. J. Bot.* **50** 1035–1045

Tepfer S S, Karpoff A J and Greyson R I 1966 Effects of growth substances on excised floral buds of *Aquilegia*; *Am. J. Bot.* **53** 148–157

Thein M M 1957 Survey of anatomical and microchemical changes in the shoot of *Xanthium pennsylvanicum* in relation to photoperiodism; *Am. J. Bot.* **44** 514–522

Tran Thanh Van M and Trinh H 1978 Morphogenesis in thin layers. Concept, methodology and results; in *Frontiers of plant tissue culture* (ed.) T A Thorpe Proc. 4th Internat. Congr. Plant Tissue and Cell Culture (Calgary) 37–48

Von Bartalanffy 1965 Zur Geschiechte theoretischer Modelle in der Biologie; *Stud. Gen.* **18** 290–298

Wadhi M 1967 Floral morphogenesis in vitro; in *Seminar on plant cell, tissue and organ culture* (ed.) B M Johri (Delhi) 85–87

Wardlaw C W 1963 Experimental investigation of floral morphogenesis in *Petasites hybridus*; *Nature (London)* **198** 560–561

Wardlaw C W 1965 The organization of the shoot apex; in *Encyclopaedia of Plant Physiology* (ed.) W Ruhland **15** 966–1076

Wilson K S and Cutter V M Jr 1955 Localization of acid phosphatase in the embryo sac and endosperm of *Cocos nucifera*; *Am. J. Bot.* **42** 116–119

Wareing P F 1978 Determination in plant development; *Bot. Mag. Tokyo Special Issue* **1** 3–17

Witsch H 1965 in *Encyclopaedia of plant physiology* (ed.) W Ruhland (Berlin: Springer-Verlag) 15

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 539-551
© Printed in India.

Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation*

SHUBHADA PATANKAR, C P JOSHI, S A RANADE, MRINAL BHAVE and P K RANJEKAR**

Biochemistry Division, National Chemical Laboratory, Pune 411 008, India

MS received 21 August 1984; revised 28 March 1985

Abstract. Using the HCl-Giemsa banding technique, the total proportion of condensed chromatin was determined by planimetry in 23 plant species and was found to vary from 14–77%. Comparison of condensed chromatin values with DNA content indicated that the latter was involved in determining the interphase nuclear structure. The actual amounts of different DNA components in these species were estimated in terms of picograms. Statistical analysis of condensed chromatin values with quantities of different types of DNA sequences showed the best correlation with highly repeated DNA sequences, suggesting that these sequences could be playing an important role in governing the species-specific chromatin condensation in plants. The amount of DNA packaged per unit length of chromatin was also shown to be a determinant of interphase nuclear structure.

Keywords. Interphase nuclear structure; condensed chromatin; DNA sequences.

1. Introduction

Higher plants, contrary to animals, show species-specific organization of chromatin within the interphase nucleus. They also exemplify several unique features such as wide variations in DNA content, absence of facultative heterochromatin and diverse kinds of DNA sequence organization (Ranjekar 1982). This poses a fundamentally interesting question; can the interphase nuclear structures observed in plants be linked with parameters such as nuclear DNA content, gross DNA architecture and relative abundance of any of the specific DNA frequency classes? Earlier reports in a few plant species (Barlow 1977; Nagl 1979a, b; Nagl and Fusenig 1979; Nagl and Bachmann 1980) have suggested that nuclear DNA content and proportion of different frequency classes of repeated DNA sequences may influence the nuclear organization. This suggestion, however, requires additional screening of a large number of species. We have, therefore, undertaken the work of heterochromatin visualization in the interphase nuclei of 23 plant species using the HCl-Giemsa banding technique (Joshi and Ranjekar 1980, 1982). Molecular analysis of the DNAs in these species has been reported earlier (Ranjekar *et al* 1974, 1976, 1978c; Seshadri and Ranjekar 1979, 1980a; Deshpande and Ranjekar 1980; Bhave *et al* 1984; Lakshmi and Ranjekar 1984; Lakshmi *et al* 1984). The present report gives a consolidated account of a relationship among the three parameters namely interphase nuclear structure, nuclear DNA content and amounts of single copy and reiterated DNA sequences.

* NCL Communication No. 3455

** To whom all correspondence should be addressed

2. Materials and methods

2.1 *Staining with HCl-Giemsa technique*

Seeds of 21 plant species listed in table 1 were germinated on moist filter paper in petri dishes. Root tips (1–2 cm) were fixed in acetic acid:ethanol (1:3) for 24 hr and then stored in 70% ethanol till further use. In case of *Allium sativum* and *A. cepa*, root tips were obtained from bulbs and then processed as above.

For air dried preparations, the root tips were squashed in a drop of 45% acetic acid after 10 min maceration in 1 N HCl at room temperature and flattened by mechanical pressure. Precaution was taken to keep the pressure uniform. Coverglasses were detached using liquid nitrogen and the slides were air dried after immersion in 90% ethanol.

HCl-Giemsa treatment was carried out according to the schedule of Joshi and Ranjekar (1980). The treatment time with concentrated HCl varied from 5–30 min. Prior to treatment with concentrated HCl, slides were incubated in distilled water for 10–15 min. After the HCl treatment, the slides were thoroughly washed with cold running water, stained in 4% Giemsa (BDH) diluted with M/15 Sorensen's phosphate buffer, pH 6·8 and mounted in DPX.

To determine the interphase nuclear structure, at least 50 nuclei from different

Table 1. Interphase nuclear structure and condensed chromatin in higher plants.

Plant species	DNA content	Nuclear structure	Number of chromocenters Mean ± SD	condensed chromatin (% nuclear area) Mean ± SD
Raphanus sativus	0·40*	Chromocentric	15 ± 0·33	14·93 ± 1·86
Phaseolus aureus	0.50*	Chromocentric	16 ± 0·50	21·77 ± 2·68
Oryza sativa	0·60*	Chromocentric	11 ± 0·50	13·80 ± 2·11
Setaria italica	0·80	Chromocentric	13 ± 0·80	14·11 ± 2·04
Luffa cylindrica	0·90*	Chromocentric	14 ± 0·50	19·59 ± 5·42
Cucumis sativus	1·00**	Chromocentric	16 ± 0·60	21·64 ± 2·59
Panicum milliare	1.00	Chromocentric	14 ± 0·75	15·58 ± 2·42
Benincasa hispida	1·10*	Chromocentric	20 ± 0·25	15·69 ± 2·63
Trichosanthes anjuina	1·10*	Chromocentric	16 ± 0·27	16·43 ± 2·06
Echinochloa frumentacea	1·30**	Chromocentric	17 ± 0·66	14·44 ± 3·13
Phaseolus vulgaris	1·30*	Chromocentric	15 ± 0·50	19·65 ± 3·33
Eleusine coracana	1·60**	Chromocentric	18 ± 0·33	15·56 ± 2·17
Phaseolus mungo	1·75	Chromocentric	13 ± 0·20	16·11 ± 3·39
Phaseolus aconitifolius	2·00	Chromocentric	16 ± 0·60	15·51 ± 1·91
Luffa acutangula	2·22	Chromocentric	15 ± 0·60	16·11 ± 2·96
Pennisetum americanum	2·45	Reticulate	—	26·82 ± 4·78
Coccinia indica	2·75	Chromocentric	14 ± 0·25	18·85 ± 1·05
Sorghum vulgare	4·60	Reticulate	—	25·08 ± 2·44
Hordeum vulgare	5·50**	Reticulate	—	60·87 ± 8·70
Secale cereale	9·50**	Reticulate	—	66·20 ± 1·96
Allium sativum	15·70*	Reticulate	—	77·28 ± 3·78
Allium cepa	16·75**	Reticulate	—	64·70 ± 4·33
Triticum aestivum	17·30**	Reticulate	—	60·76 ± 7·26

* From Bennett *et al* (1982).

** From Bennett and Smith (1976).

preparations were observed. Area of chromocenters, chromonemata and nuclei were calculated by planimetry. Condensed chromatin amount was calculated by using the following formula. In chromocentric nuclei

$$\% \text{ condensed chromatin} = \frac{\text{Area of chromocenters}}{\text{Area of nucleus}} \times 100$$

In reticulate nuclei

$$\% \text{ condensed chromatin} = \frac{\text{Area of chromonemata}}{\text{Area of nucleus}} \times 100$$

The condensed chromatin values were expressed as % nuclear area and they represented mean of at least 50 nuclei.

2.2 *Determination of* DNA *content*

DNA content was determined only in *Setaria italica*, *Panicum milliare*, *Phaseolus mungo*, *Phaseolus aconitifolius*, *Luffa acutangula*, *Pennisetum americanum*, *Coccinia indica* and *Sorghum vulgare* where the data was not available. DNA quantitation was done by using the standard two wavelength method of Patau (1952) where λ_1 selected was 550 nm. Schiff's reagent preparation (pH adjusted to 3·6) and Feulgen staining were carried out according to Darlington and LaCour (1976). To ensure the absolute amount of DNA per 2C nucleus, late anaphase or telophase figures were selected. *A. cepa* (2C = 33·5 pg) was used as standard. The 1C amounts were obtained from 2C values.

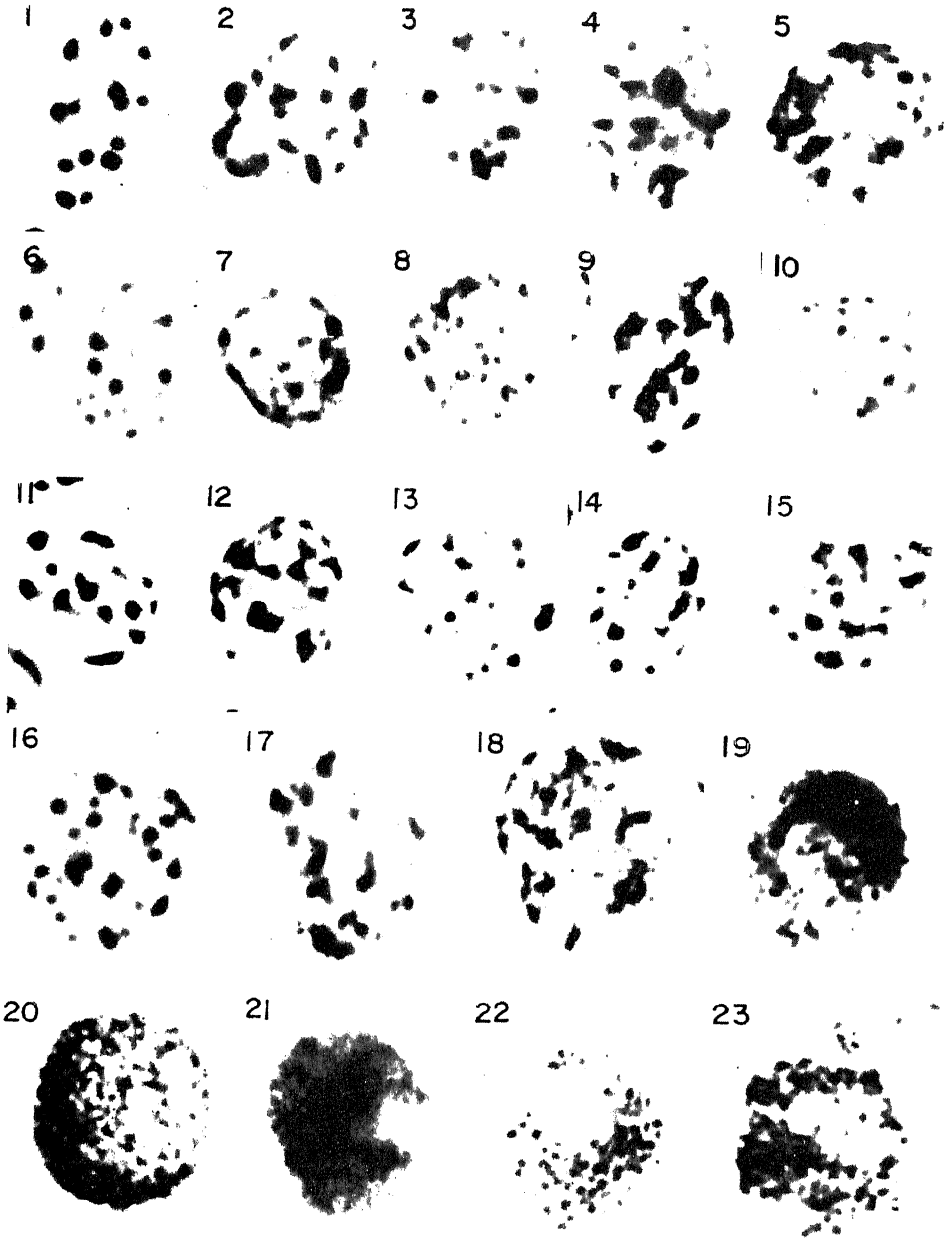
2.3 *Amounts of repetitive* DNA

From our published reassociation kinetics data, the actual amounts of different DNA components were estimated in terms of picograms. The DNA sequences forming duplexes by Cot 0·1 M × sec/l were considered to be highly repetitive. The DNA reannealing between Cot 0·1 M × sec/l and the limit repetitive Cot value was assumed to include intermediately repetitive DNA sequences. The amount of total repetitive DNA represents the sum of the amounts of highly repetitive DNA and intermediately repetitive DNA. The DNA sequences reassociating after the limit repetitive Cot were considered to be mainly unique.

3. Results and discussion

All the species under the present investigation show two types of interphase nuclear organization namely, chromocentric and reticulate. Out of 23 species, 16 species have chromocentric organization and show distinct large and dark chromocenters on a faint euchromatic background (figures 1–16). The remaining 7 species have reticulate nuclei (figures 17–23) and exhibit a dense network of chromomeres and chromonemata.

In chromocentric species, the number of chromocenters is considered to be a species-specific character. Since these chromocenters have been shown to correspond to heterochromatin (Nagl and Fusenig 1979), the condensed chromatin values as determined from chromocenters will, therefore, correspond to heterochromatin amount. These amounts as shown in table 1, vary in a narrow range of 13–21% of nuclear area. In reticulate nuclei, on the other hand, condensed chromatin corresponds



Figures 1–23. Interphase nuclei, **1–16.** showing chromocentric structure, **17–23.** showing reticulate structure. **1.** *R. sativus* (× 1400), **2.** *P. aureus* (× 10500), **3.** *O. sativa* (× 9800), **4.** *S. italica* (× 6400), **5.** *L. cylindrica* (× 7600), **6.** *C. sativus* (× 1200), **7.** *P. milliare* (× 3000), **8.** *B. hispida* (× 7250), **9.** *T. anjuina* (× 7000), **10.** *E. frumentacea* (× 2250), **11.** *P. vulgaris* (× 7800), **12.** *E. coracana* (× 8250). **13.** *P. mungo* (× 6700), **14.** *P. aconitifolius* (× 7000), **15.** *L. acutangula* (× 7000), **16.** *C. indica* (× 1700), **17.** *P. americanum* (× 8000), **18.** *S. vulgare* (× 2070), **19.** *H. vulgare* (× 1200), **20.** *S. cereale* (× 1200), **21.** *A. sativum* (× 5900), **22.** *A. cepa* (× 5000), **23.** *T. aestivum* (× 6000).

to both heterochromatin and condensed euchromatin (Nagl and Fusenig 1979) and the values, therefore, vary in a wide range of 25–77% (table 1).

The occurrence of different classes of heterochromatin among plants is well known. Furthermore from our earlier work (Joshi and Ranjekar 1982; Patankar and Ranjekar 1984a, b), it is well established that the HCl-Giemsa technique visualizes most of the types of heterochromatin as well as condensed euchromatin. The HCl-Giemsa technique, thus has the advantage over the BSG technique which visualizes only heterochromatic portions as chromocenters in both chromocentric as well as reticulate nuclei. The values obtained using HCl-Giemsa technique, therefore, though not absolute, give a fairly correct picture of condensed chromatin in plants. For example, the values obtained in *P. vulgaris* (19·65 ± 3·33) and *A. cepa* (64·70 ± 4·33) agree well with the reported values of 17% and 52% respectively, calculated by electron microscopic morphometry (Nagl *et al* 1983).

The nuclear DNA content (1C) values show a wide range of 0·4–17·3 pg (table 1). The data also clearly indicate that the chromocentric nuclei are characterized by small genome size (1C < 2 pg, except *Coccinia*) and reticulate nuclei have large genome size (1C > 2 pg). The role of nuclear DNA in determining the nuclear structure has been suggested earlier (Lafontaine 1974; Barlow 1977; Nagl and Fusenig 1979; Nagl 1982). Our data on condensed chromatin also show its strong positive dependence on the nuclear DNA content suggesting thereby a direct role of nuclear DNA content in gross chromatin organization in plant nuclei (table 4, coefficient of correlation $r = 0{\cdot}9053$) (figure 24). This also confirms the nucleotypic effect of DNA content on chromatin structure as suggested earlier by Nagl and Fusenig (1979).

Delay (1948), Lafontaine (1974) and Barlow (1977) have earlier suggested the role of chromosome size and DNA amount per chromatid in determining interphase nuclear

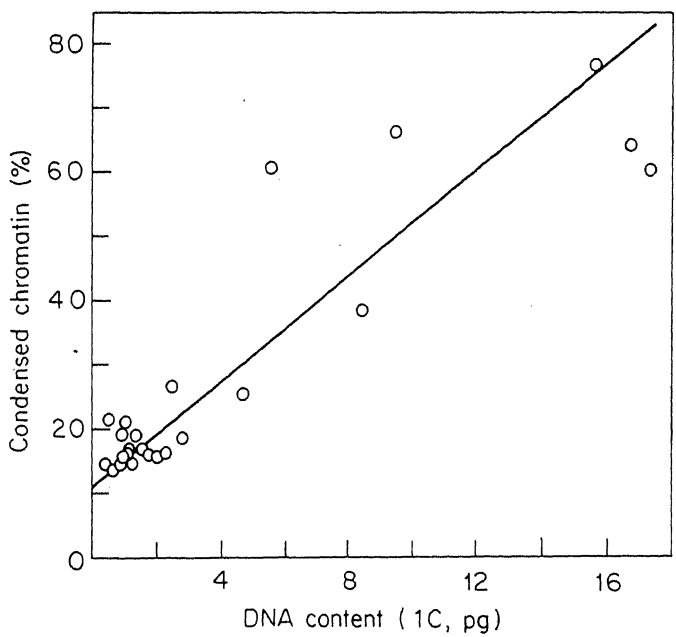


Figure 24. Relation between DNA content (1C, pg) and amount of condensed chromatin (%) in interphase nuclei of 23 plant species.

Table 2. DNA content, chromosome number and chromosome lengths in a few higher plants.

Plant species	DNA content (1C, pg)	Chromosome number (n)	Chromosome length (μ)	Total length of haploid complement (μ)	DNA/unit chromosome length (pg/μ)	Reference*
Chromocentric:						
Raphanus sativus	0·40	09	2·3–1·6	15·66	0·0255	Mukherjee (1979)
Phaseolus aureus	0·50	11	2·8–1·0	18·70	0·0267	Sarbhoy (1980)
Oryza sativa	0·60	12	1·9–1·0	28·40	0·0210	Kurata and Omura (1978)
Setaria italica	0·80	09	2·5–1·6	18·75	0·0426	Chikara and Gupta (1979)
Cucumis sativus	1·00	07	4·0–1·9	20·52	0·0487	Sen and Datta (1978)
Trichosanthes anjuina	1·10	11	3·3–0·9	30·91	0·0355	Singh and Roy (1979)
Phaseolus vulgaris	1·30	11	3·0–1·5	21·00	0·0619	Sarbhoy (1980)
Eleusine coracana	1·60	18	3·9–1·9	43·21	0·0370	Kempanna *et al* (1976)
Phaseolus mungo	1·75	11	2·2–1·0	16·60	0·1054	Sarbhoy (1980)
Reticulate:						
Hordeum vulgare	5·50	07	10·0–8·0	50·02	0·1099	Noda and Kasha (1978)
Secale cereale	9·50	07	8·0–6·0	58·32	0·1628	Vosa (1974)
Allium sativum	15·70	08	11·4–6·6	73·13	0·2146	Choudhary (1978)
Allium cepa	16·75	08	10·0–7·0	72·00	0·2326	Jones and Rees (1968)
Triticum aestivum	17·30	21	6·0–3·0	100·55	0·1720	Pegington and Rees (1970)

Note: Mean amount of DNA (pg/μ) of chromatin length is (i) 0·0449 for chromocentric nuclei, (ii) 0·1784 for reticulate nuclei.

* Reference cited is only for chromosome size and total chromatin length of haploid complement.

structure. The present data confirm the role of chromosome size where chromocentr nuclei are characterized by the small size of the chromosomes with their lengths varyin from 1–3 μ (table 2). Reticulate nuclei, on the other hand, show long chromosomes wit lengths varying from 3–4 μ or more (table 2). We further suggest that apart fro chromosome size, packaging of DNA in chromatin and chromosomes also plays a important role in determining the interphase nuclear structure. There can be limitatio on the amount of DNA packaged in the chromosome per unit length and thus th distribution of the total DNA along the chromosomes would reflect the specific nuclea structure. Data on 14 species (table 2) show that as compared to chromocentric nucl there is nearly 4 times the amount of DNA packaged per unit length of chromosomes i reticulate nuclei. The visualization of extensive network of chromomeres an chromonemata, may, therefore, be attributed to the higher density of DNA along th chromosomes.

From table 3, it can be seen that the higher plants show wide variations of 25–80 % i the amounts of repetitive DNA. From this table, it is also evident that there is an increas in unique as well as repetitive DNA with increasing DNA content. The increase i repetitive DNA is, however, about three times more than that of unique DNA (figure 25). A similar trend has been observed earlier in case of *Allium*, *Anemone*, *Lathyrus*, *Lolium* and members of family Gramineae and Compositae where the rate of increase fo repetitive DNA to non-repetitive DNA varies from 1·08 to 5·53 (Hutchinson *et al* 1980 Repetitive DNA, in general, has been suggested to play a major role in chromati condensation and thereby in interphase nuclear structure in plants (Lafontaine 1974 Nagl 1979b, 1982; Narayan and Rees 1976). Repetitive DNA is, however, highl heterogeneous and consists of different families containing sequences of varyin degrees of reiteration. To assess the role of any specific class of repeat sequences i chromatin structure, we compared the amounts of different classes of repetitive DN with condensed chromatin values. The data in tables 3 and 4 indicate a positiv correlation between total repetitive DNA and condensed chromatin ($r = 0{\cdot}8907$). Th

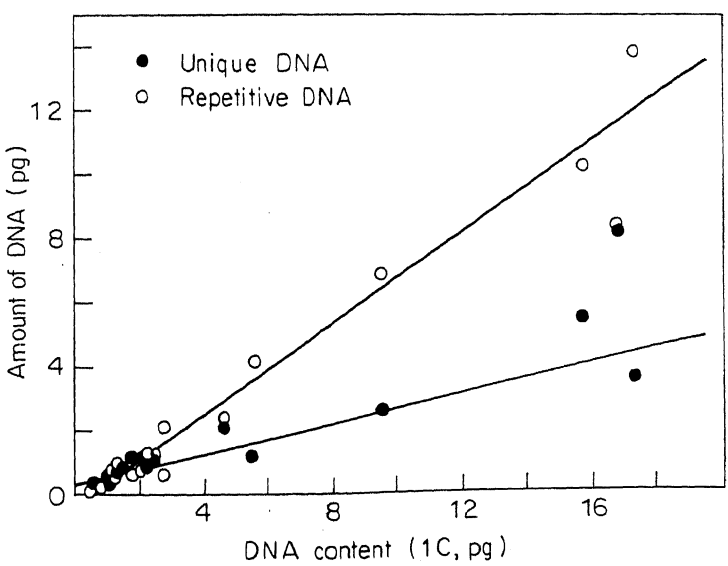


Figure 25. Relation between DNA content (1C, pg) and amount of unique DNA (pg) and tot repetitive DNA (pg) in 23 plant species.

Table 3. Relationship between repetitive DNA and condensed chromatin.

Plant species	Highly repetitive DNA		Intermediately repetitive DNA		Total repetitive DNA		Unique DNA		Condensed chromatin
	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	%	$\times 10^{-12}$ g	(% nuclear area)
Raphanus sativus	15·0	0·060	30·0	0·120	45·0	0·180	55·0	0·220	14·93
Phaseolus aureus	9·0	0·045	38·0	0·190	47·0	0·235	53·0	0·265	21·77
Oryza sativa	8·5	0·051	43·5	0·261	52·0	0·312	48·0	0·288	13·80
Setaria italica	20·0	0·160	10·0	0·080	30·0	0·240	70·0	0·560	14·11
Luffa cylindrica	11·0	0·099	40·0	0·360	51·0	0·459	49·0	0·441	19·59
Cucumis sativus	20·0	0·200	16·0	0·160	36·0	0·360	64·0	0·640	21·64
Panicum milliare	15·0	0·150	25·0	0·250	40·0	0·400	60·0	0·600	15·58
Benincasa hispida	15·5	0·170	32·5	0·357	48·0	0·527	52·0	0·573	15·69
Trichosanthes anjuina	18·0	0·198	47·0	0·517	65·0	0·715	35·0	0·385	16·43
Echinochloa frumentacea	17·0	0·221	25·0	0·325	42·0	0·546	58·0	0·754	14·44
Phaseolus vulgaris	12·0	0·156	28·0	0·364	40·0	0·520	60·0	0·780	19·65
Eleusine coracana	19·0	0·304	30·0	0·480	49·0	0·784	51·0	0·816	15·56
Phaseolus mungo	7·0	0·122	27·5	0·481	34·5	0·603	65·5	1·147	16·11
Phaseolus aconitifolius	5·0	0·100	36·0	0·720	41·0	0·820	59·0	1·180	15·51
Luffa acutangula	13·0	0·288	46·0	1·020	59·0	1·309	41·0	0·911	16·11
Pennisetum americanum	20·0	0·490	34·0	0·833	54·0	1·323	46·0	1·127	26·82
Coccinia indica	15·0	0·413	10·0	0·275	25·0	0·688	75·0	2·062	18·85
Sorghum vulgare	17·0	0·782	35·0	1·610	52·0	2·392	48·0	2·208	25·08
Hordeum vulgare	19·0	1·036	59·0	3·215	78·0	4·291	22·0	1·219	60·87
Secale cereale	23·0	2·185	50·0	4·798	73·0	6·983	27·0	2·517	66·20
Allium sativum	39·0	6·123	26·0	4·082	65·0	10·205	35·0	5·495	77·28
Allium cepa	20·0	3·350	30·0	5·025	50·0	8·375	50·0	8·375	64·70
Triticum aestivum	18·0	3·115	62·0	10·726	80·0	13·840	20·0	3·460	60·76

Table 4. Statistical analysis—Pearsonian coefficient of correlation and *t* test of significance.

Variable *X*	Variable *Y*	Pearsonian coefficient of correlation*(*r*)	Degrees of freedom** (*d.f.*)	*t* test of significance (*t*)***
1C genome content (pg)	Total unique DNA (pg)	0·8988	21	9·3939
1C genome content (pg)	Total repetitive DNA (pg)	0·9742	21	19·7687
1C genome content (pg)	Condensed chromatin (%)	0·9053	21	9·7660
Condensed chromatin (%)	Highly repetitive DNA (pg)	0·9012	21	9·5287
Condensed chromatin (%)	Intermediately repetitive DNA (pg)	0·8178	21	6·5110
Condensed chromatin (%)	Total repetitive DNA (pg)	0·8907	21	9·4440

* Pearsonian coefficient of correlation, $r = \Sigma xy/(\Sigma x^2 \cdot \Sigma y^2)^{1/2}$ where $x = X - \overline{X}$ and $y = Y - \overline{Y}$.

** Degrees of freedom *d.f.* = Number of observations $(N) - 2$.

*** *t* test of significance $t = r\sqrt{d.f./1 - r^2}$ where *r* = Pearsonian coefficient of correlation.

Standard *t* values $t_{0.01}$ and $t_{0.05}$ for *d.f.* = 21 are 2·831 and 2·080 respectively.

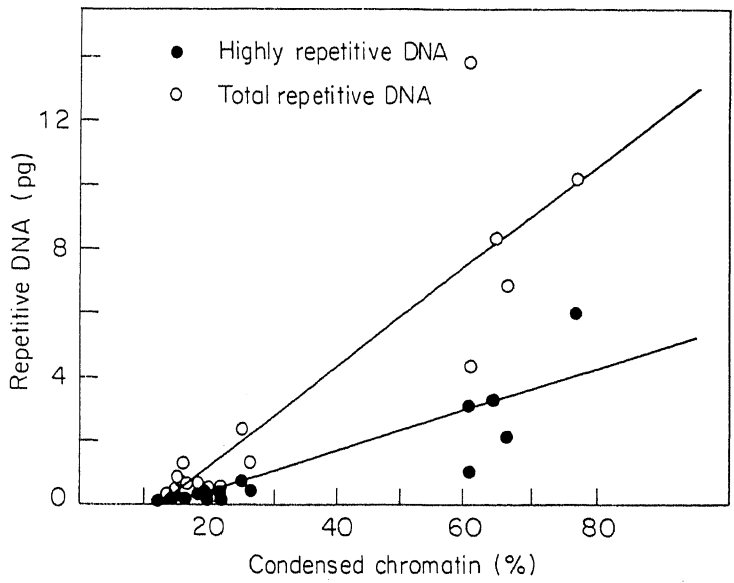


Figure 26. Relation between condensed chromatin and amounts of highly and total repetitive DNA (pg) in 23 plant species.

highest correlation is, however, seen between highly repetitive DNA and condensed chromatin ($r = 0{\cdot}9012$) (figure 26), suggesting a major role to the highly repetitive DNA rather than to the total repetitive DNA. The suggested relationship of highly repetitive DNA and/or satellite DNA with centromeric heterochromatin and chromocenters (Timmis *et al* 1975; Nagl and Capesius 1977; Appels and Peacock 1978; Narayan and Durrant 1983) supports our observation that chromatin condensation is dependent on the highly repetitive DNA. The chromatin condensation is, however, a very complex phenomenon and involves several other factors such as ionic changes, protein modifications and specific stages of development.

Table 5. Relationship between satellite DNA presence and interphase nuclear structure.

Plant species	Satellite		Reference
	Natural*	Cryptic**	
Chromocentric:			
Raphanus sativus	+	−	Ranjekar *et al* (1978b)
Phaseolus aureus	+	−	Ingle *et al* (1973)
Luffa cylindrica	+	−	Ingle *et al* (1973)
Cucumis sativus	+	−	Ranjekar *et al* (1978b)
Phaseolus vulgaris	+	−	Ingle *et al* (1973)
Phaseolus mungo	+	−	Seshadri and Ranjekar (1979)
Phaseolus aconitifolius	+	−	Seshadri and Ranjekar (1979)
Reticulate:			
Hordeum vulgare	−	+	Ranjekar *et al* (1978a)
Secale cereale	−	+	Appels *et al* (1978)
Triticum aestivum	−	+	Ranjekar *et al* (1978a)

* Natural satellite is a repetitive DNA fraction which bands in equilibrium CsCl density gradient centrifugation at a buoyant density different than that of main band DNA.

** Cryptic or hidden satellite is revealed only after binding of DNA either with antibiotics or heavy metals followed by equilibrium density gradient centrifugation in CsCl or Cs_2SO_4.

Table 6. Relationship between interspersion pattern and interphase nuclear structure.

Plant species	Interspersion pattern	Reference
Chromocentric:		
Phaseolus aureus	Intermediate	Seshadri and Ranjekar (1980b)
Oryza sativa	Absent	Gupta *et al* (1981)
Setaria italica	long	Lakshmi (1984)
Luffa cylindrica	mixed	Bhave *et al* (1985)
Panicum milliare	mixed	Lakshmi (1984)
Benincasa hispida	mixed	Bhave *et al* (1985)
Echinochloa frumentacea	mixed	Lakshmi (1984)
Phaseolus vulgaris	Intermediate	Seshadri and Ranjekar (1980b)
Eleusine coracana	mixed	Gupta and Ranjekar (1981)
Luffa acutangula	mixed	Bhave *et al* (1985)
Coccinia indica	mixed	Bhave *et al* (1985)
Reticulate:		
Pennisetum americanum	long	Gupta and Ranjekar (1982)
Sorghum vulgare	mixed	Lakshmi *et al* (1984)
Secale cereale	short	Smith and Flavell (1977)
Allium cepa	short	Stack and Comings (1979)
Triticum aestivum	short	Flavell and Smith (1976)

The data presented in table 5 show that species with chromocentric nuclei show the presence of natural satellites whereas those with reticulate nuclei have mainly cryptic satellites. Furthermore, it can be seen from table 6 that species having reticulate nuclei show, in general, predominantly typical short period interspersion pattern of genome organization while species with chromocentric nuclei have either long period or mixed interspersion patterns. Since the highly reiterated DNA (satellite) has a positive role in chromatin condensation, the above findings may have a direct bearing in determining the structural complexity of interphase nuclei in plants.

In conclusion, our data reaffirm the earlier predictions about the role of nuclear DNA content and also propose the involvement of specifically highly repetitive DNA and DNA packaging in chromatin condensation in higher plants. Further work is being undertaken to evaluate the role of highly repetitive/satellite DNAs and DNA sequence organization in chromatin condensation.

Acknowledgements

One of the authors (SP) thanks the CSIR for a fellowship. The substantial help received from Prof. A K Sharma in DNA cytophotometry is gratefully acknowledged. Research fellowships from National Council for Educational and Research and Training to SAR and MB are gratefully acknowledged.

References

Appels R and Peacock W J 1979 The arrangement and evolution of highly repeated (satellite) DNA sequences with special reference to *Drosophila*; *Int. Rev. Cytol. Suppl.* **8** 69–126

Appels R, Driscoll C and Peacock W J 1978 Heterochromatin and highly repeated DNA sequences in rye (*Secale cereale*); *Chromosoma (Berl.)* **70** 67–89.

Barlow P W 1977 Determinants of nuclear chromatin structure in angiosperms; *Ann. Sci. Nat. Bot.* (Paris) **18** 193–206

Bennett M D and Smith J B 1976 Nuclear DNA amounts in angiosperms; *Philo. Trans. R. Soc. London.* **B274** 227–274

Bennett M D, Smith J B and Heslop-Harrison J S 1982 Nuclear DNA amounts in angiosperms; *Proc. R. Soc. London* **B216** 174–199

Bhave M R, Gupta V S and Ranjekar P K 1985 Molecular analysis of cucurbitaceae genomes III: Arrangement and size distribution of repeat and single copy DNA sequences in four plant species; *Plant Syst. Evol.* (In press)

Bhave M R, Lagu M D and Ranjekar P K 1984 Molecular analysis of cucurbitaceae genomes. I: Comparison of DNA reassociation kinetics in six plant species; *Plant Sci. Lett.* **33** 127–136

Chikara J and Gupta P K 1979 Karyological studies in the genus *Setaria* I: Variability within *Setaria italica* (L) Beauv. *J. Cytol. Genet.* **14** 75–79

Choudhary A D 1978 *Mutational studies in Allium sativum Linn.* Ph.D. Thesis, Nagpur Univeristy, Nagpur, India

Darlington C D and LaCour L F 1976 *Handling of chromosomes* 6th edn (London: George Allen and Unwin)

Delay C 1948 Recherches sur la structure des noyax quiescents chez des phanerogames; *Rev. Cytol. Cytophysiol. Veg.* **10** 103–228

Deshpande V G and Ranjekar P K 1980 Repetitive DNA in three gramineae species with low DNA content Hoppe-Seyler's *Z. Physiol. Chem.* **361** 1223–1233

Flavell R B and Smith D B 1976 Nucleotide sequence organization in wheat genome; *Heredity* **37** 231–252

Gupta V S and Ranjekar P K 1981 DNA sequence organization in finger millet (*Eleusine coracana*); *J. Biosci.* **3** 417–430

Gupta V S and Ranjekar P K 1982 Genome organization in pearl millet; *Indian J. Biochem. Biophys.* **19** 167–170

Gupta V S, Gadre S R and Ranjekar P K 1981 Novel DNA sequence organization in rice genome; *Biochim. Biophys. Acta* **656** 147–154

Hutchinson J, Narayan R K J and Rees H 1980 Constraints upon the composition of supplementary DNA; Chromasoma (Berl.) **78** 137–145

Ingle J, Pearson C G and Sinclair J 1973 Species distribution and properties of nuclear satellite DNA in higher plants; *Nature (New Biol.)* **242** 193–197

Jones R N and Rees H 1968 Nuclear DNA variation in *Allium; Heredity* **25** 591–605

Joshi C P and Ranjekar P K 1980 Technique for heterochromatin visualization and chromosome banding in plants; *Nucleus (Calcutta)* **23** 169–176

Joshi C P and Ranjekar P K 1982 Visualization and distribution of heterochromatin in interphase nuclei of several plant species as revealed by new Giemsa banding technique; *Cytologia (Tokyo)* **47** 471–480

Kempanna C, Laxmi P V and Nasrath R 1976 Karyotype studies in *Eleusine coracana; Nucleus (Calcutta)* **19** 200–203

Kurata N and Omura T 1978 Karyotype analysis in rice. I. A new method for identifying all chromosome Pairs *Jpn. J. Genet.* **53** 251–255

Lafontaine J G 1974 Ultrastructural organization in plant cell nuclei; in *The cell nucleus* (ed.) H Busch (New York: Academic Press) Vol. 1 pp. 149–185

Lakshmi S 1984 *Genome characterization in plants with special reference to four millet species* Ph.D. Thesis, University of Poona, Poona, India

Lakshmi S and Ranjekar P K 1984 Novel molecular features of millet genomes; *Indian J. Biochem. Biophys.* **21** 299–303

Lakshmi S, Gupta V S and Ranjekar P K 1984 Molecular organization of great millet (*Sorghum vulgare*) DNA; *J. Biosci.* **6** 795–809

Mukherjee P 1979 Karyotype variation in ten strains of Indian radish (*Raphanus sativus* L.); *Cytologia (Tokyo)* **44** 347–352

Nagl W 1979a Condensed interphase chromatin in plant and animal cell nuclei; *Plant Syst. Evol. Suppl.* **2** 247–260

Nagl W 1979b Interphase chromatin organization in plant nuclei as determined by genome organization. *Hoppe-Seyler's Z. Physiol. Chem.* **360** 331–332

Nagl W 1982 Condensed chromatin: species specificity, tissue specificity and cell cycle specificity as monitored by scanning cytometry; in *Cell growth* (ed.) C Nicolini (New York: Plenum Publishing Corporation) pp. 171–218

Nagl W and Bachmann K 1980 Condensed chromatin in diploid and allopolyploid *Microseris* species with different genome size: A quantitative electron microscopic study; *Theor. Appl. Genet.* **57** 107–111

Nagl W and Capesius I 1977 Repetitive DNA and heterochromatin as factors of karyotype evolution in phylogeny and ontogeny of orchids; in *Chromosomes today* (eds) A de la Chapelle and M Sorsa (Amsterdam: Elsevier/North Holland Biomedical Press) Vol. 6 pp. 141–152

Nagl W and Fusenig H P 1979 Types of chromatin organization in plant nuclei; *Plant Syst. Evol. Suppl.* **2** 221–233

Nagl W, Jeanjour M, Kling H, Kuhner S, Michels I, Muller T and Stein B 1983 Genome and chromatin organization in higher plants; *Biol. Zentralbl.* **102** 129–148

Narayan R K J and Rees H 1976 Nuclear DNA variation in *Lathyrus*; *Chromosoma (Berl.)* **54** 141–154

Narayan R K J and Durrant A 1983 DNA distribution in chromosomes of *Lathyrus* species; *Genetica* **61** 47–53

Noda K and Kasha K J 1978 A modified Giemsa C-banding technique for *Hordeum* species; *Stain Technol.* **53** 155–162

Patankar S and Ranjekar P K 1984a Interphase nuclear structure and heterochromatin in *Phaseolus* plant species; *Plant Cell Rep.* **3** 130–133

Patankar S and Ranjekar P K 1984b Condensed chromatin and its under-replication during root differentiation in leguminosae; *Plant Cell Rep.* **3** 250–253

Patau K 1952 Absorption microphotometry of irregular shaped objects; *Chromosoma (Berl.)* **5** 341–362

Pegington C and Rees H 1970 Chromosome weights and measures in the triticineae; *Heredity* **25** 195–205

Ranjekar P K 1982 Analysis of plant genomes—A molecular approach; *J. Sci. Ind Res.* **41** 384–393

Ranjekar P K, Lafontaine J G and Pallota D 1974 Characterization of repetitive DNA in rye (*Secale cereale*); *Chromosoma (Berl.)* **48** 427–440

Ranjekar P K, Pallota D and Lafontaine J G 1976 Analysis of the genome of plants. II. Characterization of repetitive DNA in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*); *Biochim. Biophys. Acta* **425** 30–40

Ranjekar P K, Pallota D and Lafontaine J G 1978a Analysis of plant genomes. III. Denaturation and reassociation properties of cryptic satellite DNAs in barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*); *Biochim. Biophys. Acta* **520** 103–110

Ranjekar P K, Pallota D and Lafontaine J G 1978b Analysis of plant genomes. IV. Isolation and characterization of satellite DNA components from two dicotyledons, cucumber (*Cucumis sativus*) and radish (*Raphanus sativus*); *Can. J. Biochem.* **56** 808–815

Ranjekar P K, Pallota D and Lafontaine J G 1978c Analysis of plant genomes. V. Comparative study of molecular properties of DNAs of seven *Allium* species; *Biochem. Genet.* **16** 957–970

Sarbhoy R K 1980 Karyological studies in the genus *Phaseolus* Linn; *Cytologia (Tokyo)* **45** 363–373

Sen R and Datta K B 1978 Cytological studies in some Indian cultivated varieties of *Cucumis* L; *J. Cytol. Genet.* **13** 16–22

Seshadri M and Ranjekar P K 1979 Genome characterization of three plant species belonging to genus *Phaseolus; Indian J. Biochem. Biophys.* **16** 1–5

Seshadri M and Ranjekar P K 1980a Denaturation and renaturation properties of the genome of *Phaseolus vulgaris; Hoppe-Seyler's Z. Physiol. Chem.* **361** 1041–1048

Seshadri M and Ranjekar P K 1980b An unusual pattern of genome organization in two *Phaseolus* plant species; *Biochim. Biophys. Acta* **610** 211–220

Singh A K and Roy R P 1979 Cytological studies in *Trichosanthes* L; *J. Cytol. Genet.* **14** 50–57

Smith D B and Flavell R B 1977 Nucleotide sequence organization in rye genome; *Biochim. Biophys. Acta* **474** 82–97

Stack S M and Comings D E 1979 Chromosomes and DNA of *Allium cepa; Chromosoma (Berl.)* **70** 161–182

Timmis J N, Deumling B and Ingle J 1975 Localization of satellite DNA sequences in nuclei and chromosomes of two plants; *Nature (New Biol.)* **257** 152–155

Vosa C G 1974 The basic karyotype of rye (*Secale cereale*) analysed with Giemsa and fluorescence methods; *Heredity* **33** 403–408

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 553-559
© Printed in India.

Development of male and female gametophytes and integument initiation in *Prenanthes brunoniana* Wall. (Compositae)

SARVESH KUMAR SOOD and TEJINDER SINGH THAKUR

Department of Biosciences, Himachal Pradesh University, Simla 171 005, India

MS received 21 September 1984

Abstract. The development of gametophytes and initiation of integument are described i *Prenanthes brunoniana*. Anther wall layers consist of an epidermis, endothecium, a middl layer and tapetum. Endothecium develops fibrous thickenings. Tapetum is of secretary type Cytokinesis is of simultaneous type. Pollen tetrads are tetrahedral and are shed at the 3-celle stage. Ovule is anatropous, unitegmic and tenuinucellate. Integumentary vascular supply i present. The cells of dermatogen, subdermatogen and inner core all take part in th integument formation. Embryo sac development is of the Polygonum type.

Keywords. *Prenanthes brunoniana;* gametophytes; integument initiation; Compositae.

1. Introduction

The family Compositae is embryologically highly interesting for exhibiting grea diversity in the development and organisation of the female gametophyte, presence o synergid haustoria and peculiar behaviour of the antipodal cells (Opperman 1904 Dahlgren 1920, 1924; Harling 1951a, b; Davis 1961).

The previous embryological work on this family has been reviewed by Schnar (1931) and Davis (1966). Among the recent papers, the works of Borgen (1971) Chennaveeraiah and Patil (1971), Newcomb (1973) and Maheswari Devi and Pullaiah (1976) are significant. Embryologically the genus *Prenanthes* is unknown, and this lacuna prompted us to take up the present investigation.

2. Material and methods

P. brunoniana Wall. was collected from Fagu forest (Simla District) during Septembe to December, 1977 and fixed in formalin-acetic-alcohol. Conventional methods o microtomy were followed. Serial sections (7–14 μ) were cut and stained with safranin fast green. Pollen acetolysis was carried out using Erdtman's technique (Erdtma 1952).

3. Observations

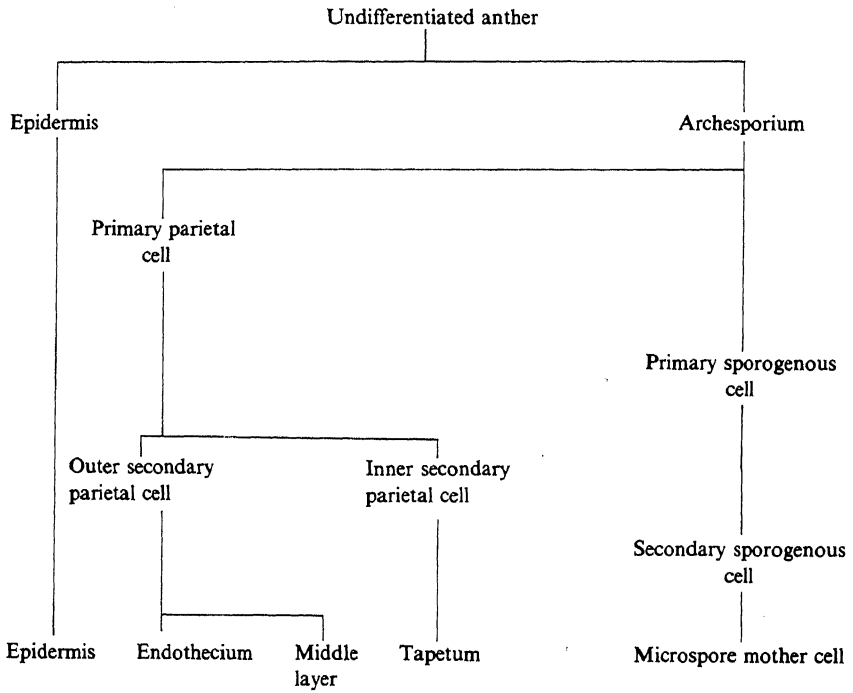
3.1 *Microsporangium, microsporogenesis and male gametophyte*

The young anther is 4-lobed (figure 1). A single hypodermal archesporial cel differentiates in each lobe (figure 2) which divides by periclinal division to form a outer primary parietal cell and an inner primary sporogenous cell (figure 3). Th primary parietal cell by periclinal and anticlinal divisions forms an outer secondar

parietal layer (*osp*) and an inner secondary parietal layer (*isp*) (figure 4). The *osp* by periclinal division forms two layers of which the outer develops into the endothecium and inner into the middle layer whereas the *isp* forms the tapetum (figure 5). Thus the fully developed anther wall shows epidermis, endothecium, single middle layer and glandular tapetum with uninucleate cells (figures 6, 9). At maturity the middle layer disorganises (figure 7) and the cells of the endothecium develop fibrous thickenings (figure 8).

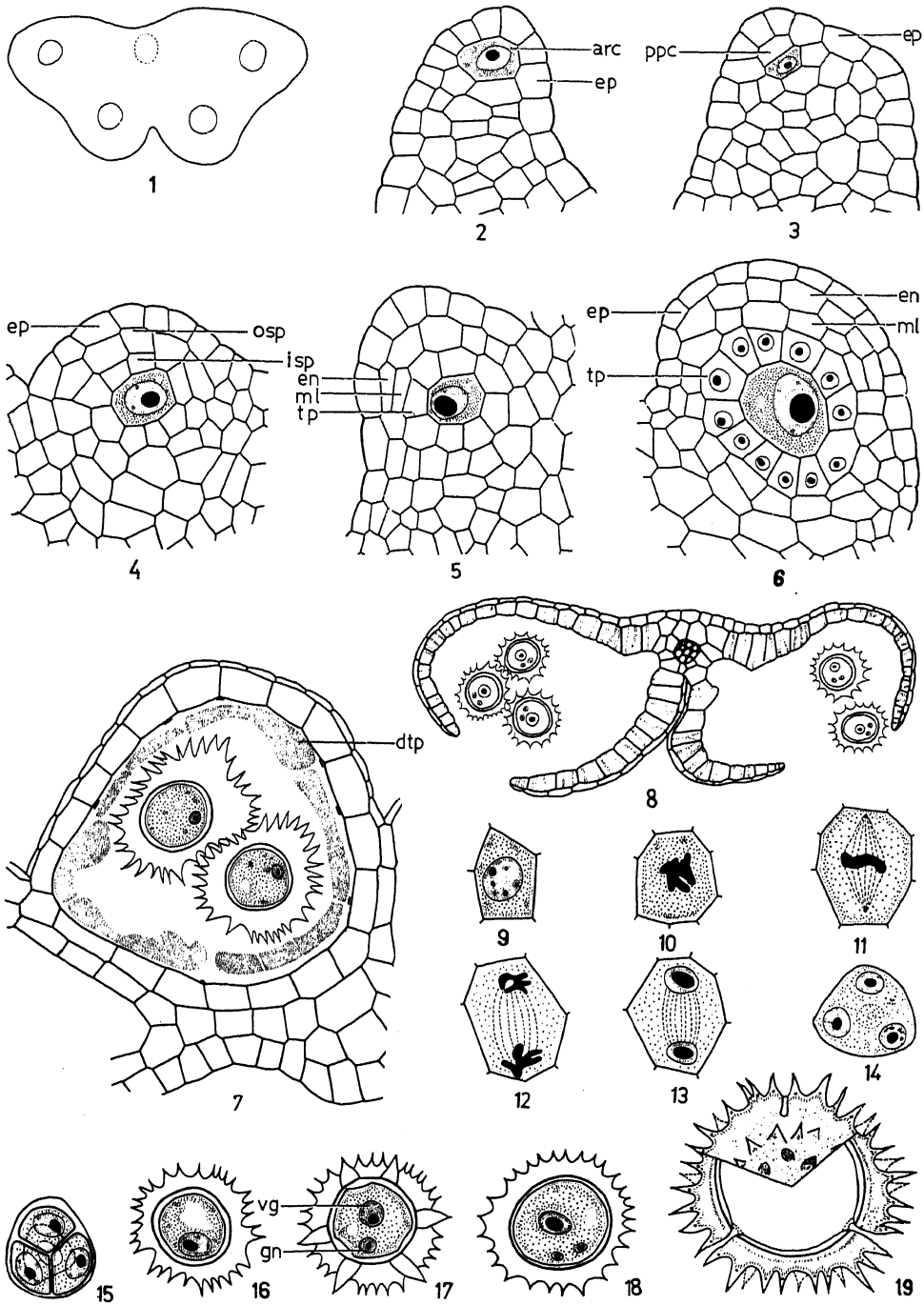
Simultaneous cytokinesis in microspore mother cells results in tetrahedral microspore tetrads (figures 10–15). The pollen nucleus (figure 16) divides to form a large vegetative and a small generative cell (figure 17). The mature pollen grain is 3-celled, tricolpate and echinolophate (figures 18, 19).

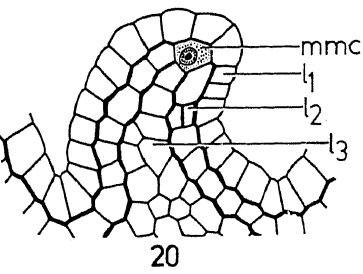
In a mature anther the adjacent lobes become confluent by the degeneration of the partition walls and dehiscence occurs by a longitudinal split (figure 8).



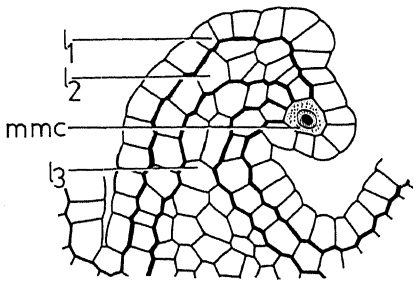
Schematic representation of the ontogeny of anther wall layers in *P. brunoniana* Wall.

Figures 1–19. Microsporangium, microsporogenesis and male gametophyte. **1.** TS of young anther (outline). **2–5.** Enlarged anther lobes showing differentiation of various wall layers and spore mother cells. **6.** Anther lobe showing nearly developed wall layers. **7.** Anther wall layers at pollen grain stage. **8.** Dehiscence of anther lobes. **9.** Tapetal cell. **10–14.** Meiotic divisions in microspore mother cell. **15.** Tetrahedral tetrad. **16–18.** 1-, 2- and 3-celled pollen grains. **19.** Palynogram. (*arc*, archesporium; *dtp*, degenerated tapetum; *en*, endothecium; *ep*, epidermis; *gn*, generative cell; *isp*, inner secondary parietal layer; *ml*, middle layer; *osp*, outer secondary parietal layer; *ppc*, primary parietal cell; *tp*, tapetum; *vg*, vegetative cell). (1 and 8, × 243; 2–7 and 16–19, × 606; 9–15, × 966).

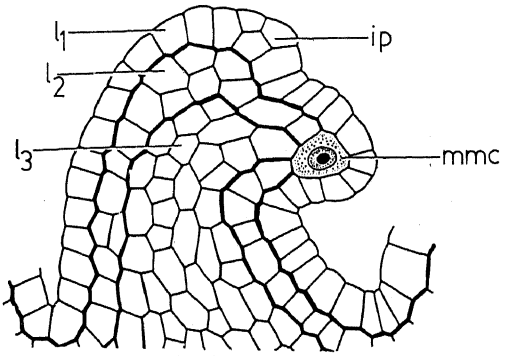

1
arc
ep
2
ppc
ep
3
ep
osp
isp
4
en
ml
tp
5
ep
en
ml
tp
6
dtp
7
8
9
10
11
12
13
14
15
vg
gn
16
17
18
19



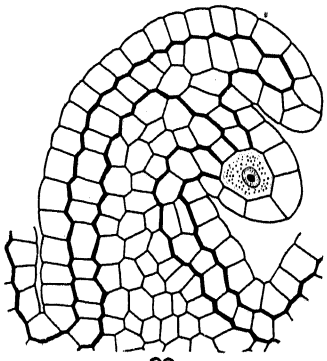
20



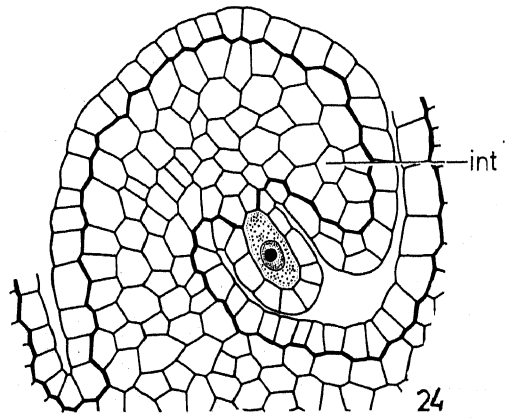
21



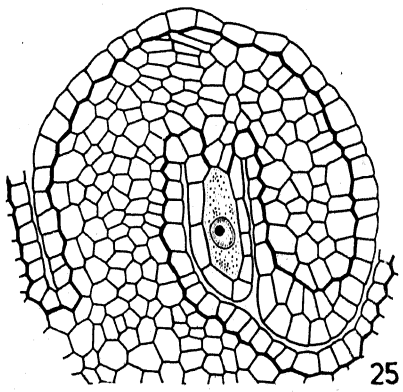
22



23



24



25

Figures 20–25. Curvature of ovule. Note the differentiation of dermatogen, subdermatogen and central core at megaspore mother cell stage. **20.** Longisection of ovule. **21–24.** Same; showing the progressive curvature of ovule and the development of integument. **25.** Anatropous ovule with massive single integument. (l_1, dermatogen; l_2, subdermatogen; l_3, inner core; *mmc*, megaspore mother cell; *int*, integument; *ip*, integument primordium) (20–24, × 333; 25, × 243).

3.2 *Megasporangium*

The basal ovular primordium shows three cell zones; dermatogen (l_1); subdermatogen (l_2) and an inner core (l_3) (figure 20). The integument initial arises as a bulge in the ovular primordium. This bulge consists of cells from the dermatogen and subdermatogen layers (figure 21). Due to the periclinal and anticlinal divisions in l_1, l_2 and l_3 layers the integument becomes large and thick and as a result grows down (figures 22–25). The innermost layer of the integument differentiates into an integumentary tapetum.

The ovule is anatropous, unitegmic and tenuinucellate at the megaspore mother cell stage (figure 25). Some of the cells lining the micropylar canal elongate radially and function as an obturator. The funicular vascular supply enters through the chalaza and extends up to the tip of the integument.

3.3 *Megasporogenesis and female gametophyte*

A single hypodermal archesporial cell (figure 26) functions directly as the megaspore mother cell (figure 27). It undergoes meiotic divisions resulting in a linear tetrad of megaspores (figure 28). The chalazal megaspore is functional (figure 29). It undergoes three mitotic divisions giving rise to two- (figure 30), four- (figure 31) and 8-nucleate (figure 32) gametophytes. The organised embryo sac has an egg apparatus, a central cell with secondary nucleus and three antipodal cells (figure 33). The mature embryo sac lies in direct contact with the endothelium due to the degeneration of the nucellus (figure 33).

4. Discussion

The anther wall is 4-layered thick and its development corresponds to the dicotyledonous type of Davis (1966). In *Lactuca sativa* (Gates and Rees 1921) the tapetal cells lying on one side of the loculus are quadrinucleate whereas those on the other side are binucleate. However, in *P. brunoniana* the tapetal cells are uninucleate on both the sides.

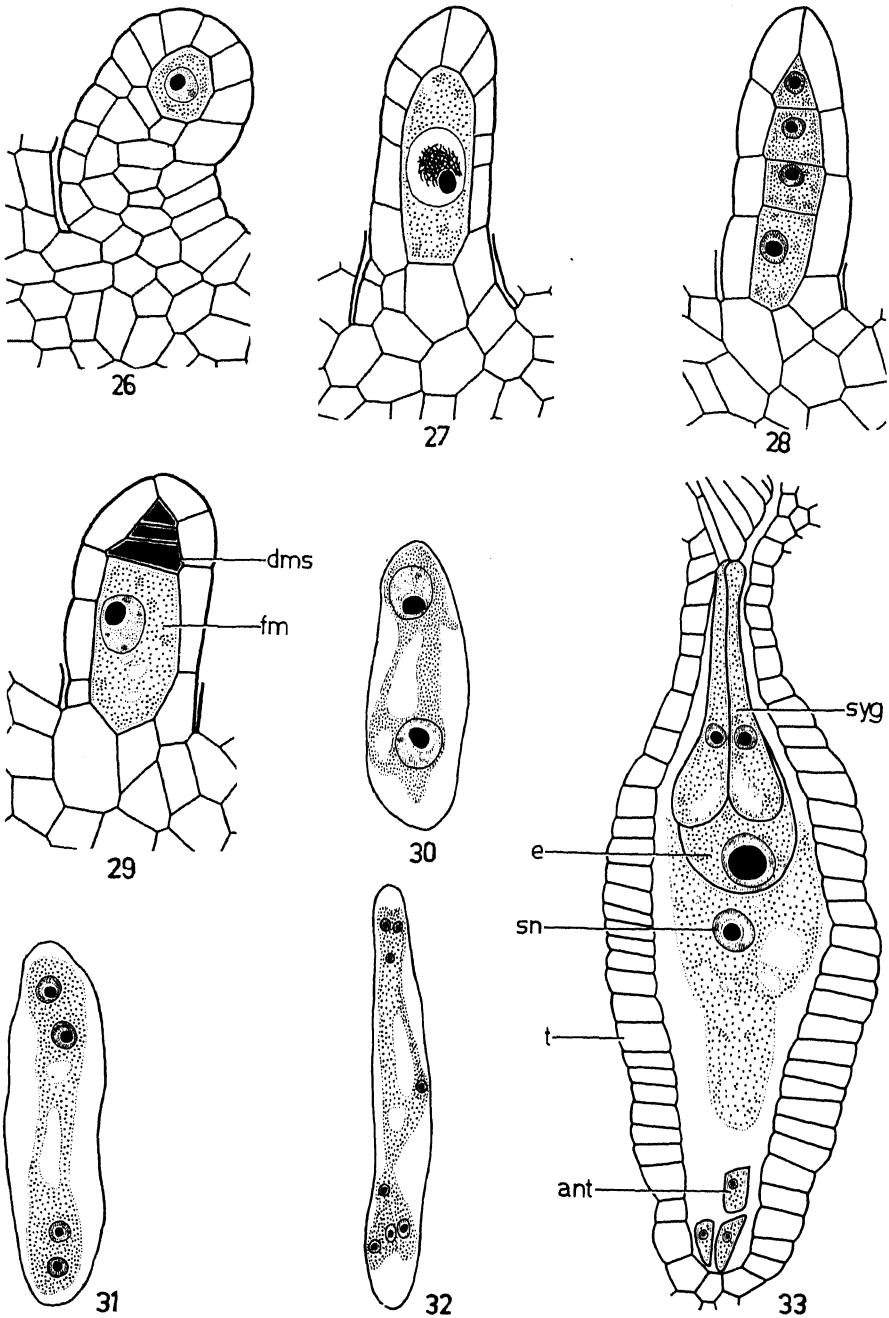
The tapetum in *P. brunoniana* is of the glandular type. This type has been reported previously only in one of the taxa of this family *i.e. Ainsliaea aptera* (Kapil and Sethi 1962).

The pollen tetrads are tetrahedral, decussate or isobilateral in Compositae (Schnarf 1931; Davis 1966). *P. brunoniana* shows only tetrahedral type of pollen tetrads.

So far there is no report on the initiation of integument in Compositae. The present study shows that the cells of dermatogen, subdermatogen and inner core all take part in the integument formation. Also the funicular vascular supply enters the chalaza and extends up to the tip of the integument whereas in *Launaea pinnatifida* (Venkateswarlu 1941) it ends at the chalaza.

The presence of obturator is not common in this family. Dahlgren (1920, 1924) reported the presence of hair-like structures in the micropylar canal of *Lactuca muralis* and *Mutisia candolleana*. The present study also shows the presence of obturator in the micropylar canal.

In *Anthemis, Chrysanthemum, Erigeron* and *Tridax* (Harling 1951a, b; Hjelmqvist 1951; Maheshwari and Roy 1952) mono-, bi- and tetrasporic types of embryo sac is



Figures 26–33. Megasporogenesis and female gametophyte. **26.** Longisection of young ovule showing archesporial cell. **27.** Same; showing megaspore mother cell. **28.** Linear tetrad of megaspores. **29.** Functional megaspore. **30–32.** Two-, 4 and 8-nucleate embryo sacs. **33.** Organised embryo sac (*ant*, antipodals; *dms*, degenerated megaspores; *e*, egg; *fm*, functional megaspore; *sn*, secondary nucleus; *syg*, synergid; *t*, integumentary tapetum). (26–32, × 606; 33, × 333).

present. However, in *P. brunoniana,* as in most of other Compositae members, monosporic type of embryo sac is present.

Synergid haustoria are reported in *Calendula* and *Ursinia* (Dahlgren 1920, 1924). In *P. brunoniana*, they are absent.

Acknowledgements

The authors are grateful to Prof. H O Agrawal, Himachal Pradesh University, for his interest in this study and to Drs P R M Rao and A S Rao, Nagarjuna University, Guntur, for help in various ways.

References

Borgen L 1971 A new type of tetrasporic embryo sac in *Chrysanthemum ferulaceum* (Webb) Sunding-Norw; *J. Bot.* **18** 169–175

Chennaveeraiah M S and Patil R M 1971 Apomixis in *Blumea; Phytomorphology* **21** 71–76

Dahlgren K V O 1920 Zur embryologie der Kompositen mit besonderer beriicksichtigung der endospermbildung; *Z. Bot.* **12** 481–576

Dahlgren K V O 1924 Studien uber die endospermbildung der Kompositen; *Svensk. bot. Tidskr.* **18** 177–203

Davis G L 1961 The life-history of *Podolepis jaceoides* (Sims) Voss-II. Megasporogenesis, female gametophyte and embryogeny; *Phytomorphology* **11** 206–219

Davis G L 1966 *Systematic embryology of the angiosperms* (New York: John Wiley)

Erdtman G 1952 *Pollen morphology and plant taxonomy, angiosperms (An introduction to palynology I)* (Massachusetts: Waltham)

Gates R R and Rees E M 1921 A cytological study of pollen development in *Lactuca; Ann. Bot.* **16** 123–148

Harling G 1951a Embryological studies in the Compositae; *Acta Hortic. Berg.* **16** 1–56

Harling G 1951b Embryological studies in the Compositae; *Acta Hortic Berg.* **16** 73–160

Hjelmqvist H 1951 The embryo sac development of *Tridax trilobata; Bot. Not.* **103** 180–187

Kapil R N and Sethi S Bala 1962 Gametogenesis and seed development in *Ainsliaea aptera* DC; *Phytomorphology* **12** 222–234

Maheshwari P and Roy S K 1952 The embryo sac and embryo of *Tridax procumbens* Linn.; *Phytomorphology* **2** 245–252

Maheswari Devi H and Pullaiah T 1976 Embryological investigations in the Melampodinae. I. *Melampodium divaricatum; Phytomorphology* **26** 77–86

Newcomb W 1973 The development of the embryo sac of sunflower *Helianthus annuus* L. before fertilization; *Can. J. Bot.* **51** 863–878

Opperman M 1904 A contribution to the life-history of *Aster; Bot. Gaz.* **37** 353–362

Schnarf K 1931 *Verleichende embryologie der angiospermen* (Berlin: Gebrüder Borntraeger)

Venkateswarlu J 1941 Vascular supply in the ovules of some Compositae; *Curr. Sci.* **10** 367–368

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 561-566
© Printed in India.

Significance of seed coat pattern in *Vigna radiata* var. *sublobata*

S IGNACIMUTHU and C R BABU
Department of Botany, University of Delhi, Delhi 110007, India

MS received 16 April 1984

Abstract. Variations in the microtopography of seed coat in eight natural populations of *V. radiata* var. *sublobata* inhabiting the Palney Hills of Western Ghats (Tamil Nadu) were investigated with the help of SEM. The seed coat patterns are population specific, and the different patterns can be conveniently classified into two types. It appears that the polymorphism in seed coat patterns is of genetic origin and is the consequence of adaptation to spatio-temporal environments.

Keywords. Seed coat pattern; *Vigna radiata* var. *sublobata;* polymorphism.

1. Introduction

During recent years seed biology has attracted the attention of biologists not only because of its importance in elucidating the evolutionary dynamics of plant populations (Stebbins 1974; Harper 1977), but also due to its application in agriculture, forestry and horticulture (Roberts 1972). Scanning electron microscopy has been extensively used to study the seed topography (Brisson and Peterson 1976), and the comparative data so obtained have been employed for a variety of purposes—solving systematic problems, identification of hybrids from segregating progenies, understanding the dormancy and associated physiological phenomena, testing seed quality and understanding the structural variations associated with pest and disease resistance (Raina 1971; Mayer and Poljakoff–Mayber 1975; Newanze and Horber 1976; Linskens *et al* 1977; Horber 1978; Craford and Kayleena 1979; Canne 1979; Rajendra *et al* 1979; Yi-Chun Hsiao and Chuang 1981). Studies on the seed coat polymorphism at the population level are rather limited (Sharma *et al* 1977).

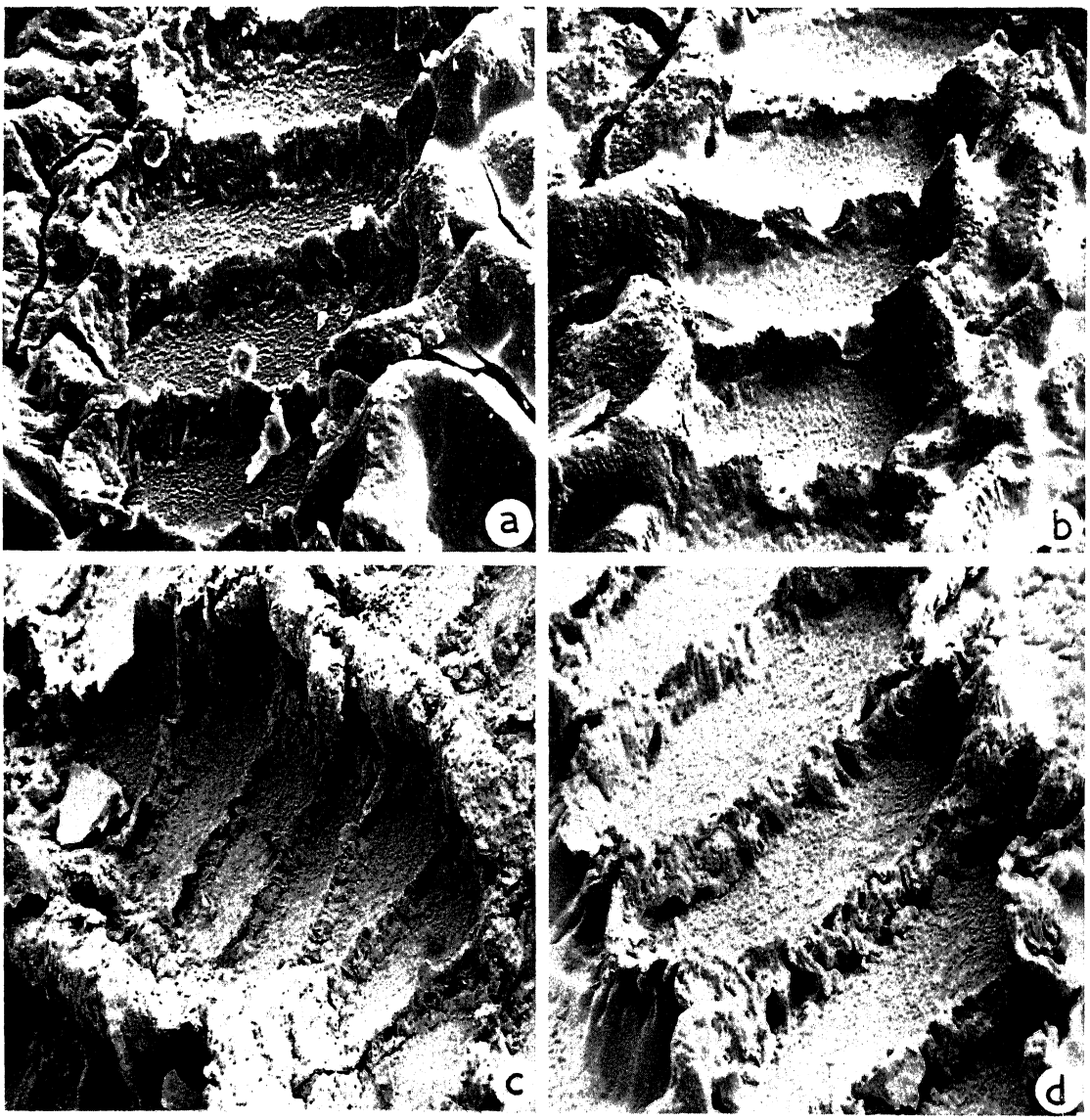
Vigna radiata var. *sublobata* (Roxb.) Verdc. is the putative progenitor of *V. mungo* (L.) Hepper, and probably of *V. radiata* (L.) Wilczek, the two major Asian pulse-yielding legumes, and is widely distributed in mountainous tracts of western, eastern and central India. Population genetic analysis of natural populations inhabiting different ecogeographical regions demonstrate that the populations are genetically well-differentiated (Ignacimuthu 1981). The present paper deals with the variations in seed coat microtopography of natural populations of *V. radiata* var. *sublobata* inhabiting the Palney range of Western Ghats, and explains the systematic significance of seed coat polymorphism.

2. Material and methods

Seed lots from eight natural populations of *Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) Verdc. (designated as V_1–V_8) inhabiting the different ecozones of Palney Hills

of Western Ghats in Tamilnadu (India) were collected and were used as experimental materials. From each of these air-dried seed lots, turgid seeds (3–4) were selected at random, and were processed for scanning. In order to assess the extent of intrapopulation variability, if any, seeds from 3 to 5 randomly selected phenotypes within the population were also scanned.

Seeds were cleaned in an ultrasonic cleaner for 10 min to remove all extraneous material. The cleaned seeds were fixed to the aluminium specimen stubs using silver paste. The stubs with fixed seeds were placed on the revolving disc of an Edward's vacuum evaporation unit. Each seed was uniformly coated with 200–300 Å thick gold. The portion of the exposed surface just below the hilum was scanned in all seed



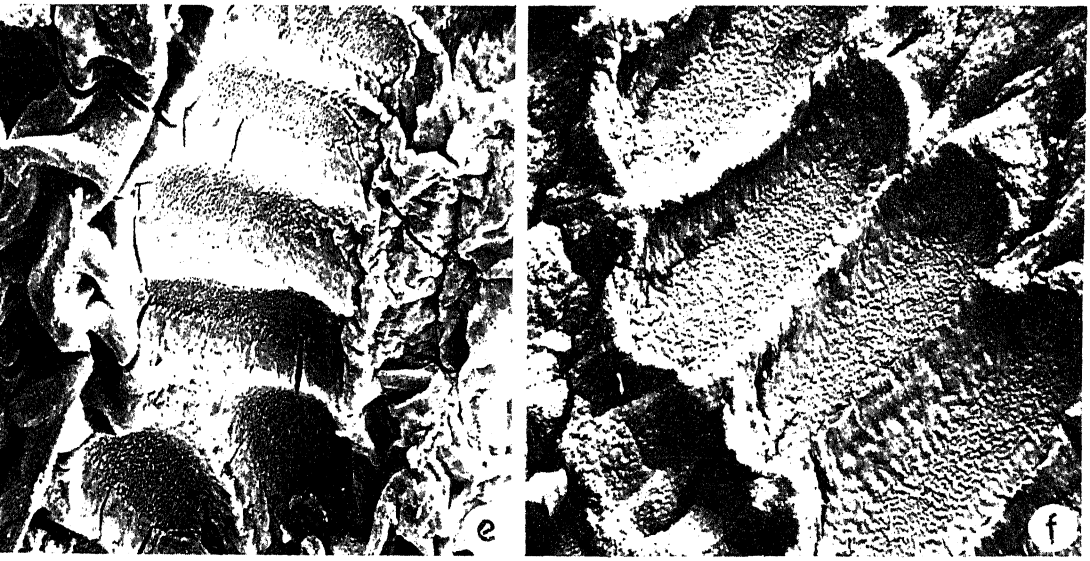


Figure 1. SEM photographs of testa from seeds of natural populations of *Vigna radiata* var. *sublobata*. Seed coat patterns (a – f × 792): **a, b.** in two phenotypes of population V_2 (Type I–subtype II), **c, d.** in two phenotypes of population V_7 (Type I–subtype II), **e.** in V_1 (Type I–subtype I), **f.** in V_2 (Type I–subtype II).

samples, at a constant angle of 45° tilt and accelerating potential of 10 kV, on an SEM (S_4 10 model, Cambridge). The micrographs were taken at different magnifications.

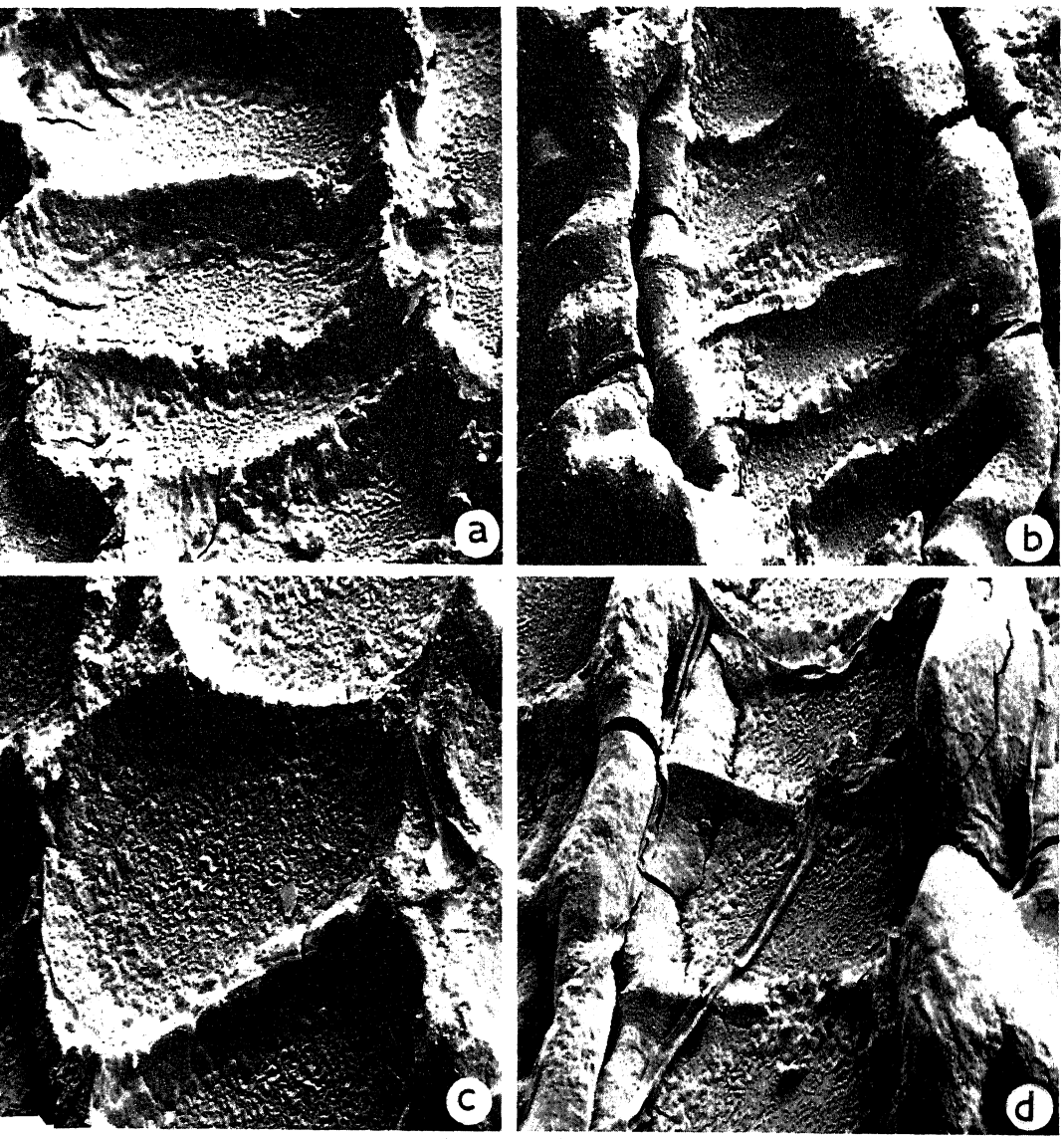
3. Observations and discussion

Except in population V_5, in all other populations the seed coat was composed of hexagonal cells arranged in horizontal rows, with longitudinal sutures (that separate different rows) and horizontal rows (that separate epidermal cells from one another) having varying degree of prominence and differential ornamentation. Within the populations, no variability was observed (figures 1a–d) suggesting that the diversity in seed coat patterns between populations is of genetic origin rather than an environmentally induced one. The genetic control of seed coat patterns has been well-established (Hadfield and Calder 1935; Lamprecht 1940).

Based on the shape of epidermal cells and their arrangement, the nature of lumen, the prominence of transverse and longitudinal sutures, and the degree and sculpture of depositions along sutures, the different patterns can be conveniently classified into two types: Type I is characterised by hexagonal epidermal cells arranged in tiers, with prominent transverse and longitudinal sutures having heavy depositions, and Type II includes the pattern which is recognizable by triangular, plano-convex epidermal cells without prominent longitudinal sutures, and with light and smooth depositions along boundaries. The microtopography of seed coat in V_5 exemplifies Type II pattern (figure 2c). The seed coat patterns of all other populations belong to Type I, within which two subtypes can be recognized. Subtype I is represented by V_1 wherein the cell lumen is

convex and pustulate, the transverse sutures are faint, and the longitudinal sutures are massive and fissured (figure 1e); and subtype II includes V_2, V_3, V_4, V_6, V_7 and V_8, and is recognizable by concave lumen of epidermal cells and prominent transverse and longitudinal sutures with heavy depositions.

In V_2 the cell lumen is shallow because of raised, prominent longitudinal and horizontal sutures, and is pustulate; the depositions are heavy and rugged in appearance (figure 1f). The pattern of V_3 is similar to that of V_2, except that the depositions along the sutures are smooth, and the contour of cells is slightly obliterated (figure 2a). In V_4 and V_6 the contours of epidermal cells are obliterated because of faint transverse sutures and massive, heavy depositions along longitudinal sutures. The



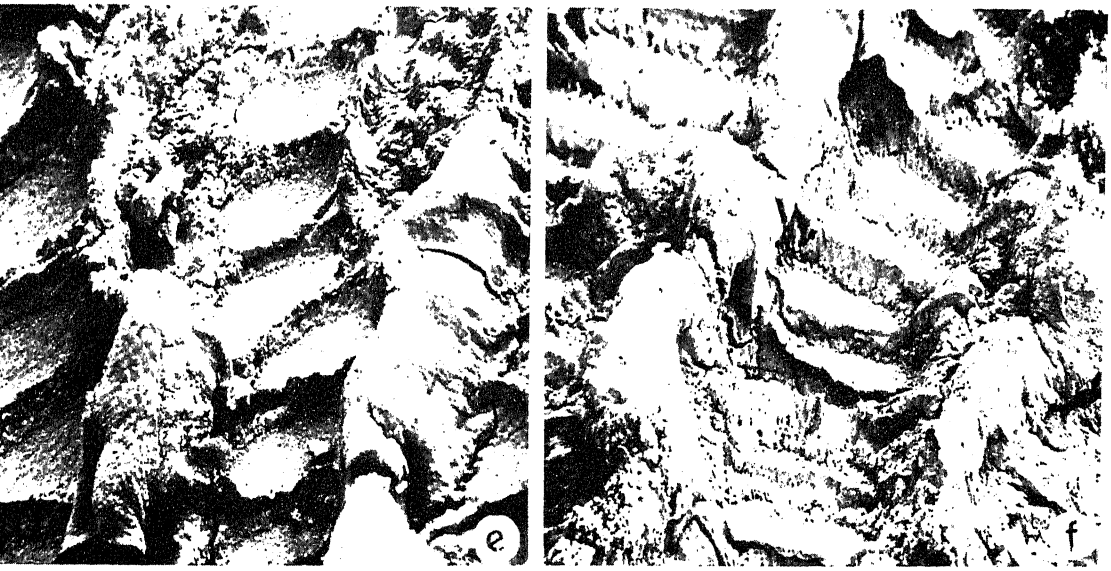


Figure 2. SEM photographs of testa from seeds of natural populations of *Vigna radiata* var. *sublobata*. Seed coat patterns (a – f × 792): **a.** in V_3 (Type I–subtype II), **b.** in V_4 (Type I–subtype II), **c.** in V_5 (Type II), **d.** in V_6 (Type I–subtype II), **e.** in V_7 (Type I–subtype II), **f.** in V_8 (Type I–subtype II).

longitudinal sutures appear as bars with heavy, smooth depositions which show irregular fissures (figures 2b, d). A similar pattern was observed in V_7 and V_8 but the topography in V_7 is more rugged because of the irregular outline of longitudinal and transverse sutures and the depositions are heavy along the longitudinal sutures and are moderate along the transverse sutures (figure 2e); in V_8 the contours of cells are completely obliterated (figure 2f).

The seed coat patterns are population specific, suggesting that the populations are well-differentiated, a point that has also been emerged as a result of the analysis of phenotypic variability (Ignacimuthu 1981). Population V_5 is distantly related to all other populations and has been recognized by taxonomists as a distinct species—*Vigna bourneae* Gamble (Verdcourt 1970). It is interesting to note that this taxon constitutes the extreme end of the variation spectrum of seed coat patterns as well as of phenotypic traits in the entire complex—*mungo-sublobata-bourneae*. V_2 appears to be more close to V_5 than to any other population and forms a connecting link between *V. sublobata* and *V. bourneae*. The fact that the seed coat patterns of the populations inhabiting Palney Hills are different from those growing in the Maharashtra range of Western Ghats (Sharma *et al* 1977) implies the role of spatio-temporal environments in the evolution of seed coat polymorphism. The Palney Hills constitute the eastward offshoot tip of the Western Ghats, and are separated by a distance of 1000 km from the Maharashtra range which form the western end of the Ghats.

References

Brisson J D and Peterson R L 1976 A critical review of the use of SEM in the study of seed coat. Workshop on plant science applications of SEM, Proceedings (IIT Research Institute, Chicago) **2** 477–495

Canne J M 1979 A light and SEM study of seed morphology in *Agalinis* (Scrophulariaceae) and its taxonomic significance; *System. Bot.* **4** 281–296

Craford D J and Kayleena E 1978 The affinities of *Chenopodium flabellifolium* (Chenopodiaceae): Evidence from seed coat surface and flavanoid chemistry; *Brittonia* **30** 313–318

Hadfield J W and Calder R A 1935 A genetic analysis of the seed coat characters, wrinkled, dimpled and smooth in *Pisum; J. Agric. Sci.* **25** 246–277

Harper J L 1977 *Population biology of plants* (London: Academic Press)

Horber E 1978 Resistance to pests of grain legumes. In *Pests of grain legumes: Ecology and control* (eds) S R Singh, H F Van Emden and T A Taylor (London, New York, San Francisco: Academic Press) 282–295

Ignacimuthu S 1981 *Investigations on genetical and biochemical variations in Phaseolus sublobatus Roxb. (Leguminosae-Papilionoideae)*. M. Phil. Thesis, University of Delhi, India

Lamprecht H 1940 Zur Genetic von *Phaseolus vulgaris* XVII–XVIII; *Hereditas* **26** 292–304.

Linskens H F, Pfahler P L and Knuiman E L 1977 Identification of soybean cultivars by the surface relief of the seed coat; *Theor. Appl. Genetics* **50** 147–150

Mayer A M and Poljakoff-Mayber A 1975 The germination of seeds. International Science Monographs; *Pure Appl. Biol.* **5** (Oxford: Pergamon Press)

Newanze K F and Horber E 1976 Seed coats of cowpeas affect ovipositional and larval development of *Callosobruchus maculatus*; *Environ. Entomol.* **5** 213–218

Raina A K 1971 Comparative resistance to the species of chickpea (*Cicer arietinum* Linn.); *J. Stored Prod. Res.* **7** 213–216

Rajendra B R, Mujeeb K A and Bates D S 1979 Genetic analysis of seed coat types in interspecific *Vigna* hybrids via SEM; *J. Hered.* **70** 245–249

Roberts E H 1972 *Viability of seeds* (London: Chapman and Hall)

Sharma K, Babu C R, Johri B M and Hepworth A 1977 SEM studies on seed coat patterns in *Phaseolus mungo-radiatus-sublobatus* complex; *Phytomorphology* **27** 106–111

Stebbins G L 1974 *Flowering plants: Evolution above the species level* (London: Edward Arnold)

Verdcourt B 1970 Studies in the Leguminosae–Papilionoideae for the flora of Tropical East Africa IV; *Kew Bull.* **24** 507–569

Yi-Chun Hsiao and Chuang T L 1981 Seed coat morphology and anatomy in *Collomia* (Polemoniaceae); Am. J. *Bot.* **68** 1155–1164

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 567-574
© Printed in India.

Survey of some Indian soils for laccase producing fungi and their lignin degrading ability

D S ARORA and D K SANDHU

Department of Biology, Guru Nanak Dev University, Amritsar 143 005, India

MS received 16 May 1984; revised 18 October 1984

Abstract. One hundred and fifty soil samples collected from three climatologically different sites were screened for laccase production using lignin-guaiacol agar plates. All the 12 fungi isolated excepting one gave positive test for qualitative lignin degradation. Five fungi were selected further for quantitative study of wood/lignin degradation. The basidiomycetes proved to be better laccase producers and decomposers of wood/lignin. These gave comparable results to a known white-rot fungus, *Polyporus versicolor* (L.) Fr. and degraded wood sawdust causing a total weight loss of 7-8% and lignin loss up to 10% in 30 days.

Keywords. Laccase; lignin; wood; soils.

1. Introduction

Laccase was first detected in the latex of *Rhus vernicifera* Brandis and later in the fruiting bodies of many basidiomycetes (Fahraeus *et al* 1958). The development of Bavendamm test (Bavendamm 1928) was of considerable significance for differentiating white- and brown-rot fungi. The former are characterized by the positive test for phenoloxidase (laccase) whereas the latter lack such activity. Since then, various methods have been evolved to detect laccase production and attempts made to establish correlation with lignin degradation (Davidson *et al* 1938; Fahraeus 1949; Nobles 1958; Kirk and Kelman 1965; Harkin and Obst 1973). Sundman and Nase (1971) found a good conformity between Bavendamm's test and lignin degradation during their studies on 52 wood-rotting and soil fungi. However, the test was not applicable to soil inhabiting ascomycetes and fungi imperfecti. The lignin component has to be removed completely or partially to increase the efficiency of lignocellulosic residues to be used as feed for ruminants, for useful conversion to sugars, alcohols or other organic solvents, or as a raw material for paper industries (Kirk and Harkin 1973; Zadrazil 1977, 1980; Higuchi 1982). The biochemistry of ligninolysis is not completely understood, however, laccase has been considered to be one of the important enzymes. It plays a key role in ligninolysis by causing its demethylation (Kirk and Chang 1975). Its involvement has been fairly substantiated by genetic studies in which laccase-less mutants were unable to degrade lignin (Ander and Eriksson 1976). Microbiology of lignin degradation has revealed fungi (particularly the white-rot basidiomycetes) to be an important group of organisms though certain brown-rot and soft-rot fungi and bacteria can cause ligninolysis to a limited extent (Ander and Eriksson 1978). Till now a number of fungi have been tested for laccase production and lignin degradation. However, no study has been undertaken to directly isolate such fungi from decomposing matter which might yield better lignin degrading species. Keeping this in view a survey of soil samples collected from three different regions was conducted to isolate laccase producing fungi.

To validate their correlation with lignin degradation the fungi were further tested for the latter process both qualitatively and quantitatively.

2. Materials and methods

2.1 *Collection and processing of soil samples*

One hundred and fifty soil samples were collected from three climatologically different sites *viz* subtropical plains of Amritsar, the south-eastern tropical islands of Andaman and hills of Mussoorie (50 samples from each site). The soils of Amritsar are solonized arid brown, saline alkali, light textured sandy loam; of Andaman saline marshy and greyish brown and of Mussoorie forest and hilly. The soil (25 g) was taken in polyethylene bags from sites where some woody matter or leaf litter decomposition occurred. The pH and moisture content of different soil samples varied from 6·5–9·5 and 10–40% respectively. The samples were stored at 4°C till further processing.

Each soil sample (1 g) was suspended in 20 ml of sterilized distilled water in a 100 ml conical flask. The flasks were agitated on a rotary shaker for 30 min and allowed to settle. The supernatant was plated by pour plate method using lignin-guaiacol medium. Four replicates/samples were plated and incubated at 25 °C for a maximum of 10 days.

2.2 *Screening of laccase producing fungi*

Phenolic compounds get oxidized to coloured quinonic products by phenoloxidase (laccase) produced particularly by the white-rot fungi. In the present study production of reddish brown colour under or around the colony was recorded as a positive test for laccase. Each fungus was isolated and subcultured on yeast glucose medium. These were further tested for colour production on lignin and guaiacol media separately and for Bavendamm reaction (Bavendamm 1928) using tannic acid as the substrate. To find any correlation between laccase production and lignin degradation the fungi isolated were tested qualitatively for the latter phenomenon according to Sundman and Nase (1971).

2.3 *Laccase production in lignin-malt extract broth*

Hundred ml conical flasks, each containing 25 ml of lignin-malt extract broth were sterilized and after inoculation were incubated at 25 ± 1°C as stationary cultures for 10 days. Triplicate flasks were processed for each fungus. The contents of each flask were filtered through Whatman filter paper No. 1 and the filtrate was centrifuged at 8500 g for 30 min at 4°C. The supernatant thus obtained was used for estimating laccase using guaiacol as the substrate (Sandhu and Arora 1984). Five ml of the reaction mixture containing 3·9 ml acetate buffer (10 mM, pH 5), 1 ml guaiacol (1·76 mM) and 0·1 ml enzyme extract were incubated at 25°C for 2 hr and the absorbance was read at 450 nm. In the blank, guaiacol was substituted by buffer. The formation of coloured products was taken as indicative of laccase activity which was expressed in relative terms as colorimetric units per ml of the enzyme.

2.4 *Degradation of wood sawdust*

Five fungal isolates including *Allescheriella crocea* (Montagne) Hughes, *Moniliella* sp., two basidiomycetes and one unidentified fungus (24 And) were selected to study their

wood/lignin degrading ability since these gave comparatively better laccase yield in broth. Two gram (dry weight) of angiospermic wood sawdust moistened with 15 ml of 0·5 % malt extract taken in 100 ml flasks were sterilized and inoculated. Uninoculated substrate served as the control. After 30 days of incubation at 25 ± 1°C triplicate set of flasks for each fungus were processed. Ten ml of acetate buffer (10 mM, pH 5) was added to each flask and after shaking on rotary shaker for 20 min the contents were filtered through Whatman filter paper No. 1. These were dried to a constant weight and weight loss caused by the organism was worked out with reference to uninoculated substrate. The residual matter was homogenized thoroughly and used for lignin estimation (Effland 1977) and the filtrate was used for laccase estimation.

2.5 *Media*

Different media used for the above studies include lignin-guaiacol medium: 1 g lignin (indulin AT) dissolved in 10 ml dioxan suspended in 1 litre of mineral salt solution was supplemented with 0·2 % malt extract and 0·4 ml guaiacol. The medium was solidified with 15 g agar. Lignin and guaiacol media were prepared separately in a similar way without adding guaiacol and lignin respectively. However, the concentration of lignin was doubled and no dioxan was used in case of lignin medium employed for Sundman's test. Lignin-malt extract broth medium contained 0·4 % lignin and 0·5 % malt extract in mineral salt solution. The composition of the latter was KH_2PO_4, 0·5 g; $MgSO_4 \cdot 7H_2O$, 0·2 g; $NaNO_3$, 0·1 g; KCl, 0·1 g; $FeSO_4$, 0·02 g; $CaNO_3$, 0·05 g; distilled water, 1 litre.

All the media and wood sawdust were sterilized by autoclaving at 15 lb pressure for 20 min. The pH value of different media before autoclaving was in the range of 5–5·5. The inoculum used for different experiments consisted of 4 mycelial discs (4 mm diam)/flask obtained from the fungal cultures grown on yeast glucose agar plates for 8–10 days. For control studies known brown and white-rot fungi were used for different tests.

2.6 *Identification of the fungi isolated*

Various fungi isolated were identified from their colonial morphology and microscopic study. *Allescheriella crocea*, *Gilmaniella humicola* Barron and *Moniliella* sp. were identified with the help of literature available (Barron 1972; Ellis 1971, 1976). Basidiomycetes were identified on the basis of clamp connections. The identification was confirmed from Commonwealth Mycological Institute (CMI), England. However, a few cultures could not be identified in our laboratory or at CMI.

3. Results

Out of 150 soil samples screened only 12 gave positive results to yield the same number of laccase producing fungi (table 1). The fungi isolated include two genera of basidiomycetes and three of fungi imperfecti *viz*, *Allescheriella crocea*, *Gilmaniella humicola* and *Moniliella* sp. while the remaining could not be identified. In general, all the cultures were able to cause colouration in lignin and guaiacol media separately. However, the colour intensity was maximum in lignin-guaiacol followed by guaiacol and lignin medium. *Allescheriella crocea* and an unidentified strain failed to cause

Table 1. Laccase producing fungi isolated from different soils.

Place of collection	Culture number	Fungi
Amritsar	13 Asr	Basidiomycete
	20 Asr	NI*
	42 Asr	*Gilmaniella humicola*
Andeman	1 And	NI
	3 And	*Moniliella* sp.
	6 And	NI
	8 And	NI
	23 And	NI
	24 And	NI
	37 And	*Allescheriella crocea*
Mussooree	61 Mus	NI
	78 Mus	Basidiomycete
Total number of samples tested		150
Number positive		12

* NI = Not identified

colouration in guaiacol whereas 24 And had no effect in lignin medium (table 2). All the white-rot fungi included as control gave positive results while the brown-rot fungi gave negative test on the three media.

3.1 *Bavendamm and Sundman's test*

All the twelve fungi isolated from soil and the white-rot fungi were positive for both the tests except for a single isolate (24 And) which gave negative Sundman test for lignin degradation but showed positive Bavendamm reaction. Both the brown-rot fungi gave negative results (table 2).

3.2 *Laccase production in lignin-malt extract broth*

All the fungi were able to produce the enzyme in broth culture to variable levels except 20 Asr, 61 Mus and a brown-rot fungus, *Poria monticola* Murrill (table 3). The two basidiomycetes gave good enzyme production, comparable to *Polyporus versicolor* (1·1 CU).

3.3 *Degradation of sawdust*

Of the five fungi selected to study wood/lignin degradation only the basidiomycetes 13 Asr and 78 Mus caused sufficient weight loss of 8·2% and 7·5% with a respective lignin loss of 10·4% and 9·4% over a period of 30 days. Though the loss was less as compared to that caused by *P. versicolor* it was higher than the rest of the fungi (table 4) which could cause a lignin loss of only 2–5%. Both the basidiomycetes ranked close to *P. versicolor* in laccase production.

Table 2. Laccase production on lignin-guaiacol, lignin and guaiacol media by fungi isolated from soil and their performance for Bavendamm and Sundmans test (lignin degradation).

Culture No.	Type of fungus	Colour production on Lignin guaiacol	Lignin	Guaiacol	Bavendamm test	Sundman test
Fungi isolated from soil						
37 And	*Allescheriella crocea* (Montagne) Hugh	+	+	−	+	+
42 Asr	*Gilmaniella humicola* Barron	+	+	+	+	+
3 And	*Moniliella* sp.	+	+	+	+	+
13 Asr	Basidiomycete	+	+	+	+	+
78 Mus	Basidiomycete	+	+	+	+	+
20 Asr	NI*	+	+	+	+	+
1 And	NI	+	+	−	+	+
6 And	NI	+	+	+	+	+
8 And	NI	+	+	+	+	+
23 And	NI	+	+	+	+	+
24 And	NI	+	−	+	+	−
61 Mus	NI	+	+	+	+	+
White-rot fungi						
7 FRI	*Daedalea flavida* Lev.	+	+	+	+	+
824 FRI	*Fomes annosus* (Fr.) Cooke	+	+	+	+	+
PAU	*Pleurotus ostreatus* (Jacq.) Fr.	+	+	+	+	+
983 FRI	*P. sajor-caju* (Fr.) Singer	+	+	+	+	+
534 FRI	*Polyporus hirsutus* Fr.	+	+	+	+	+
970 FRI	*P. sanguineus* Klotzsch	+	+	+	+	+
165 FRI	*P. versicolor* (L.) Fr.	+	+	+	+	+
Brown-rot fungi						
528 FRI	*Polyporus palustris* Berk. Curt.	−	−	−	−	−
180c FRI	*Poria monticola* Murrill	−	−	−	−	−

* NI = Not identified

4. Discussion

The use of lignin-guaiacol as the test substrate for detecting laccase producing fungi gave an advantage over the earlier studies in which guaiacol alone (Boidin 1951; Kirk and Kelman 1965) or spraying the lignin agar plates with guaiacol after fungal growth (Westermark and Eriksson 1974) has been used to detect the enzyme production. The study helped to detect colonies which might have gone unnoticed in guaiacol (*Allescheriella crocea*, 1 And) or lignin medium alone (24 And). However, *Allescheriella crocea* and 1 And were able to produce colouration in lignin-guaiacol medium which may indicate their requirement for lignin polymer to induce laccase production which then reacts more sharply with guaiacol.

The study revealed a good consistency with Bavendamm test and may provide a good substitute for screening laccase producing fungi. With some exceptions, the fungi giving positive Bavendamm reaction are considered as white-rot fungi capable of lignin degradation (Bavendamm 1928; Davidson *et al* 1938; Nobles 1951; Kirk and Kelman

Table 3. Laccase production by fungi isolated from soil in lignin malt extract broth medium.

Fungi	Laccase activity* (CU/ml)
Basidiomycete (78 Mus)	1·25
Polyporus versicolor	1·10
Basidiomycete (13 Asr)	1·04
Allescheriella crocea	0·225
Moniliella sp.	0·217
NI (6 And)	0·157
NI (1 And)	0·125
NI (23 And)	0·107
NI (8 And)	0·07
NI (24 And)	0·05
Gilmaniella humicola	0·05
NI (61 Mus)	0
NI (20 Asr)	0
Poria monticola	0

* The enzyme activity has been expressed in relative terms as colorimetric units/ml of the enzyme extract.

Table 4. Decay of angiospermic wood sawdust by different fungi after 30 days of incubation.

Fungi	Per cent loss		Laccase activity (CU/ml)
	Total weight	Lignin	
Polyporus versicolor	10·69	13·75	0·06
Basidiomycete (13 Asr)	8·16	10·40	0·06
Basidiomycete (78 Mus)	7·50	9·40	0·05
Allescheriella crocea	*+0·30	5·36	0
NI (23 And)	+0·25	3·69	0
Moniliella sp.	+0·25	2·68	0

* +represents the increase in total weight

1965; Sundman and Nase 1971). Our studies corroborate the above observations as all the fungi isolated gave positive test for qualitative lignin degradation except isolate No. 24 And. Thus it resembles *Aureobasidium pullulans* (de Bary) Arrand in giving good Bavendamm reaction but negative test for lignin degradation (Sundman and Nase 1971).

The quantitative laccase production and lignin degradation studies corroborate the earlier observations where basidiomycetes have been shown to be better enzyme producers as well as lignin decomposers as compared to fungi imperfecti (Eslyn *et al* 1975; Norris 1980; Haider and Trojanowski 1981). The inability of two isolates (20 Asr, 61 Mus) to give quantitative enzyme yield may be due to cell bound nature of the

enzyme which is not excreted into the medium but on coming in contact with the substrate in solid medium causes colouration (Lindeberg 1948; Rosenberg 1980). The slight increase in overall weight of sawdust in some fungi may be due to the fact that the fungal biomass was enough to balance the low weight loss or utilization of various wood components. No enzyme could be detected in the extract of wood decayed by *Allescheriella crocea*, *Moniliella* sp. and 23 And which may be attributed to the low level of the enzyme which gets further diluted during the processing and thus goes undetected.

Acknowledgements

The authors thank Westvaco Chemical Division, USA and Dr P S Rehill, Forest Research Institute, Dehradun, India for providing indulin AT and the fungal strains respectively. The help provided by Commonwealth Mycological Institute, England is also gratefully acknowledged. DSA further extends his thanks to UGC, New Delhi for financial assistance.

References

Ander P and Eriksson K E 1976 The importance of phenoloxidase activity in lignin degradation by the white-rot fungus *Sporotrichum pulverulentum*; *Arch. Microbiol.* **109** 1–8

Ander P and Eriksson K E 1978 Lignin degradation and utilization by microorganisms; *Prog. Indian Microbiol.* **14** 1–58

Barron G L 1972 *The genera of hyphomycetes from soil* (New York: Robert E Krieger Publishing Company)

Bavendamm W 1928 Uber des vorkommen und den Nachweis von oxydasin bei Holzzerstorenden Pilzen; *Ze. Pflanzenkrankheiten und Pflanzenschutz.* **38** 257–276

Boidin J 1951 Recherche de la tyrosinase et de la laccase chez les Basidiomycetes en culturepure. Milieuse differentiels. Interret Systematique; *Rev. Mycol.* **16** 173–197

Davidson R W, Campbell W A and Blaisdell D J 1938 Differentiation of wood decaying fungi by their reactions on gallic and tannic acid medium; *J. Agric. Res. (USA)* **57** 683–695

Effland M J 1977 Modified procedure to determine acid insoluble lignin in wood and pulp; *Tappi* **60** 143–144

Ellis M B 1971 Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England

Ellis M B 1976 More dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England

Eslyn W E, Kirk T K and Effland M J 1975 Changes in the chemical composition of wood caused by six soft-rot fungi; *Phytopathology* **65** 473–476

Fahraeus G 1949 On the oxidation of phenolic compounds by woodrotting fungi; *Ann. R. Agric. Coll. Sweden* **16** 618–629

Fahraeus G, Tullander V and Ljungren H 1958 Production of high laccase yields in cultures of fungi; *Physiol. Plant* **11** 631–643

Haider K and Trojanowski J 1981 A comparison of the degradation of ^{14}C-labelled DHP and corn stalk lignins by micro- and macro-fungi and by bacteria in *Lignin biodegradation: Microbiology, chemistry and potential applications* (eds) T K Kirk, T Higuchi and H Chang (Boca Raton, Florida: CRC Press) Vol 1 pp. 111–134

Harkin J M and Obst J R 1973 Syringaldazine an effective reagent for detecting laccase and peroxidase in fungi; *Experientia* **29** 381–387

Higuchi T 1982 Biodegradation of lignin: Biochemistry and potential applications; *Experientia* **38** 159–166

Kirk T K and Chang H M 1975 Decomposition of lignin by white-rot fungi. II. Characterization of heavily degraded lignins from decayed spruce; *Holzforschung* **29** 56–64

Kirk T K and Harkin J M 1973 Lignin biodegradation and the bioconversion of wood. Forest products and the environment; *Alche. Symp. Ser.* **69** 124–134

Kirk T K and Kelman A 1965 Lignin degradation as related to the phenoloxidases of selected wood decaying basidiomycetes; *Phytopathology* **55** 739–745

Lindeberg G 1948 On the occurrence of polyphenoloxidases in soil inhabiting basidiomycetes; *Physiol. Plant* **1** 196–205

Nobles M K 1958 A rapid test for extracellular oxidase in cultures of wood inhabiting hymenomycetes; *Can. J. Bot.* **36** 91–99

Norris D M 1980 Degradation of ^{14}C-labelled lignins and ^{14}C-labelled aromatic acids by *Fusarium solani; Appl. Environ. Microbiol.* **40** 376–380

Rosenberg S L 1980 Patterns of diffusibility of lignin and carbohydrate degrading systems in wood-rotting fungi; *Mycologia* **72** 798–812

Sandhu D K and Arora D S 1984 Laccase production by *Polyporus versicolor* on different substrates; *Acta Biotechnol.* **4** 49–57

Sundman V and Nase L 1971 A simple plate test for direct visualization of biological lignin degradation; *Pap. Puu.* **53** 67–71

Westermark U and Eriksson K E 1974 Carbohydrate dependent enzymic quinone reduction during lignin degradation; *Acta Chem. Scand.* **B28** 204–208

Zadrazil F 1977 The conversion of straw into feed by basidiomycetes; *Eur. J. Appl. Microbiol. Biotechnol.* **4** 273–281

Zadrazil F 1980 Conversion of different plant waste into feed by basidiomycetes; *Eur. J. Appl. Microbiol. Biotechnol.* **9** 243–248

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 575-582
© Printed in India.

Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils

R KASTURI BAI* and P N RAJU

Centre for Advanced Studies in Botany, University of Madras, Madras 600 005, India

* Present address: School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625 021, India

MS received 23 November 1983; revised 26 March 1985

Abstract. Immunogenic analyses were carried out for the rhizobial isolates collected from South Indian soils using the extracellular polysaccharide as the antigen. They are immunogenic eventhough their immunogenic nature was not established unequivocally in the previous studies. There was wide variation in their monosaccharide composition which could not be correlated with serological reactions. IR studies of these exopolysaccharides reveal that there are two major functional groups, amide and carboxyl in most of the isolates. The immunodiffusion analysis revealed that there are four distinct bands common to all the isolates. The antisera of the isolates D_2, D_9, V_2, V_3, Sa_1 and P_1 showed an additional hazy line.

Keywords. Rhizobial isolates; monosaccharide composition; infrared analysis; immunogenicity; extra-cellular polysaccharides.

1. Introduction

The capsular and extra-cellular polysaccharides constitute important immunological determinants in bacteria (Wilkinson 1958; Liu 1961; Sutton and Williams 1970). The noteworthy discovery of Heidelberger and Avery (1962) that carbo-hydrates are indeed of no less significance than proteins for the immunological specificity of bacteria and of bacterial types indicates that the existence of a great number of specific polysaccharides is after all as intelligible as the enormous multiplicity of proteins. The antigenic sites in serological reactions depend upon the chemical composition and properties of the component sugars mainly aminosugars and 2-acetamido-2-deoxy D-Glucose (Wilkinson 1958; Wagner *et al* 1973).

There is no unequivocal evidence for the immunogenicity of purified exopolysaccharides of rhizobia (Vincent 1977). Humphrey and Vincent (1959, 1965) on the basis of their studies on the exopolysaccharide of *R. trifolii* concluded that it is not antigenic. However, Dudman's (1964) studies on the extra-cellular soluble antigens of some of the strains of *R. melitoti* have proved the antigenicity of the extra-cellular polysaccharide fraction. There is practically little mention in literature on serological studies on rhizobia using the extra-cellular polysaccharide as the immunogen. On the basis of the IR spectral studies of the important functional groups of carbohydrates, Gouldon and Sharpe (1958) have reported serological and physiological differences among the species of the genus *Lactobacillus*.

The analysis of the monosaccharide pattern of extracellular polysaccharides of the rhizobial isolates under study indicates there is a wide variation. The present investigation was undertaken to prove the immunogenicity of the extra-cellular

polysaccharides, to see whether any correlation if at all, exists between the monomers, functional groups and the specificity of serological reactions.

2. Material and Methods

The history of the collection of the rhizobial isolates and authentication against standard hosts are given in table 1. The special synthetic medium of Zevenhuizen (1971) was used for the exopolysaccharide production of these isolates. Isolation and purification of the extra-cellular polysaccharides was done following the method of Bailey *et al* (1971) and deproteinized several times by the method of Sevag *et al* (1938) to exclude the protein completely from the polysaccharide.

2.1 *Chromatographic analysis of exopolysaccharides for their monomers*

Known quantity of the polysaccharide was dried, hydrolysed at 121°C for 1 hr with 6N HCl and then evaporated to dryness *in vacuo*. The residue was dissolved in water and was desalted (Block *et al* 1963) using anionic exchange resin (Amberlate IRA-410) and a cationic exchange resin (Amberlate IRC-50) and the clear supernatant was used for chromatographic analysis which was carried out according to the method of Zweig and Whitaker (1971) and Hepper (1972). Unidimensional chromatograms were run with ethylacetate: pyridine: water (12:5:4 v/v) as the irrigating solvents and sprayed with aniline pthalate reagent for producing a colour. The spots were identified with co-chromatograms of the respective sugars with standards. Quantitative estimation of sugars was carried out by eluting the coloured spots with 50% acetone and read at 490 nm with the respective sugars as the standard.

2.2 *Sample preparation for* IR *analysis*

About 2 mg calculated as glucose equivalents of polysaccharides was dried for 1 hr and powdered. It was then made into a fine thin film with potassium bromide (IR grade) by grinding them together. The spectra were taken in a Beckman IR-20 IR spectrophotometer.

Table 1. Details of the rhizobial isolates selected for study.

Isolate name and original host	Place of collection	Test host showing best nodulation
D_1 *Dolichos lab-lab*, Linn.	Kanjangad	*Vigna sinensis*, (L) Savi
D_2 *Dolichos lab-lab*, Linn.	Bijapur	*Dolichos lab-lab*, Linn.
D_9 *Dolichos lab-lab*, Linn.	Coimbatore	*Dolichos lab-lab*, Linn.
A_1 *Arachis hypogaea*, Willd.	Cuddalore	*Vigna sinensis*, (L) Savi
A_2 *Arachis hypogaea*, Willd.	Ponneri	*Vigna sinensis*, (L) Savi
V_2 *Vigna sinensis*, (L) Savi	Mettupalayam	*Vigna sinensis*, (L) Savi
V_3 *Vigna sinensis*, (L) Savi	Salem	*Vigna sinensis*, (L) Savi
Sa_1 *Sesbania aegyptiaca*, Pers.	Maduravoyal	*Vigna sinensis*, (L) Savi
Sa_2 *Sesbania aegyptiaca*, Pers.	Tirunelveli	*Vigna sinensis*, (L) Savi
Cg_1 *Canavalia gladiata*, DC	Shenkottai	*Vigna sinensis*, (L) Savi
P_1 *Phsaeolus trilobus*, Air.	Chepauk (Univ. Campus)	*Phaseolus mungo*, Linn

2.3 *Immunization schedule*

Before starting the injection schedule, the test animals were bled and the serum was tested with the exopolysaccharide antigens of the rhizobial isolates and found to be free of antibodies. Known quantity of the purified exopolysaccharide was dried, ground well in a mortar and dissolved in 2 ml of physiological saline to get a homogeneous solution to the final concentration of 2 mg/ml. It was mixed with an equal volume of incomplete adjuvant Arlacil, and the mixture was made into an emulsion and injected into the rabbits intramuscularly at 5-day intervals up to the 40th day when a maximum titre could be got. The schedule was started with 5 mg of exopolysaccharide, gradually increased to 7·5 mg and 10 mg in the subsequent injections and in total, 72·5 mg of exopolysaccharide was injected for each isolate. For control, the animals were given only the physiological saline and the adjuvant. The animals were bled for antisera 5 days after the last injection though the marginal earveins and the antisera were stored at $-10°C$.

2.3a *Immunodiffusion analysis on agar gel:* Agar gel was prepared using 0·9 % of Agar-Agar serva in 0·01 M sodium phosphate buffer, pH 7·0 and 0·85 % of sodium chloride. Sodium azide was aded as a preservative and the immunodiffusion studies were carried out in transparent plastic petri plates. Each antiserum was tested against its homologous polysaccharide antigen and also against antigen A and B simultaneously. The lower three walls all contain the same antiserum of the respective isolate (figure 3).

2.3b *Antigen A*: Rhizobial cells collected from 72 hr cultures were washed with sodium phosphate buffer repeatedly to remove the gum and flagella. They were machanically disrupted in a mortar in 0·85 % physiological saline. This suspension with appropriate dilution was used as antigen A and prepared for all the isolates of the present study.

2.3c *Antigen B*: Cells collected from 72 hr old liquid cultures were washed thoroughly with the buffer to remove the gum and the flagella and then ground well with a acid washed sand and the physiological saline. The suspension was centrifuged at 5000 g for 20 min and the supernatant was used as antigen B and prepared for all the isolates of the present study.

3. Results

3.1 *Monomers of extra-cellular polysaccharides*

There is qualitative and quantitative variation in the monosaccharide pattern of the extra-cellular polysaccharides. In general, on acid hydrolysis, galactose and glucose are the predominant sugars in all the rhizobial isolates occurring in varying concentrations (figure 1, table 2) except the isolate Sa_2. Only three isolates had uronic acids in their polysaccharides. In general, the concentration of glucose was higher than that of galactose in all the isolates (table 2). Methyl pentose was present in five of the isolates namely A_1, V_3, Sa_2, Cg_1, and P_1.

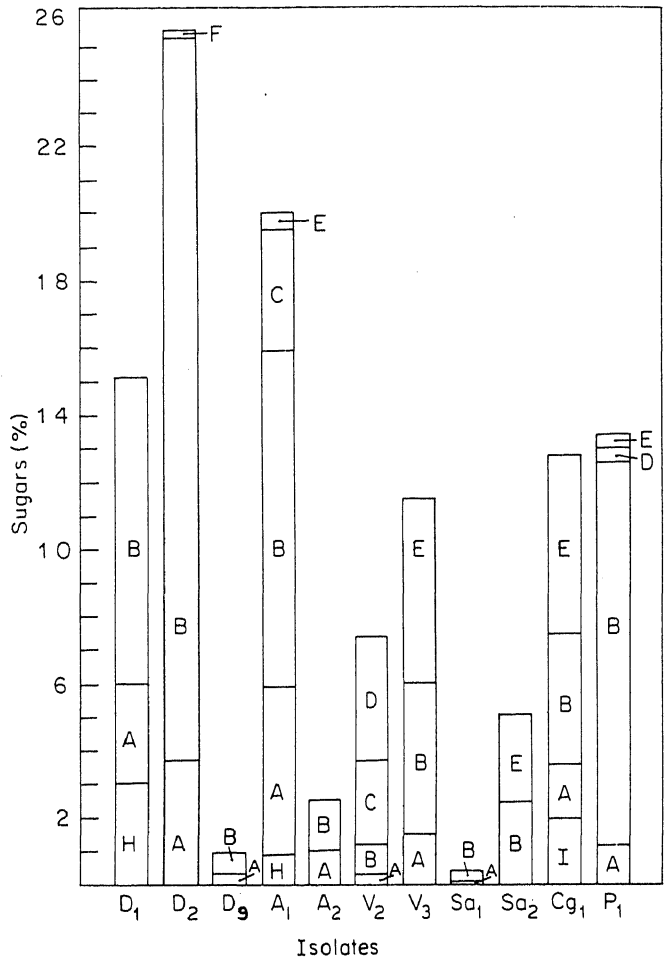


Figure 1. Quantitative analysis of the monosaccharide composition of extracellular polysaccharides of rhizobial isolates. A—Galactose. B—Glucose. C—Arabinose. D—Xylose. E—Rhamnose. F—Mannose. H—Galacturonic acid. I—Glucuronic acid.

3.2 *IR studies*

The exopolysaccharide of all the isolates except P_1 yielded two similar peaks at wave numbers 1570 cm^{-1} (6·45 μ) and 1420 cm^{-1} (7·1 μ) (figure 2) showing the presence of amide and carboxyl groups respectively. The isolates D_9, A_1 and V_2 have different type of spectrum above 8·5 μ indicating their dissimilarity in chemical structure (Levine *et al* 1953). The peaks at 6·45 μ and 7·1 μ were not seen in the isolate P_1 which is unique in having the characteristic absorption bands beyond 8·5 μ.

3.3 *Immunodiffusion reactions*

The exopolysaccharide of all the isolates were immunogenic as evidenced by the induction of antibodies in rabbits. Most of the isolates showed four distinct precipitin

Table 2. Monosaccharide composition of extra-cellular polysaccharides in μg/10 mg of polysaccharide.

Isolates	Galactose	Glucose	Calacturonic acid	Glucoronic acid	Xylose	Arabinose	Rhamnose	Mannose	Total
D_1	300	909	300	–	–	–	–	–	1509
D_2	370	2000	–	–	–	–	–	20	2390
D_9	27	55	–	–	–	–	–	–	82
A_1	500	1000	90	–	–	360	50	–	2000
A_2	100	150	–	–	–	–	–	–	250
V_2	28	90	–	–	370	250	–	–	738
V_3	150	450	–	–	–	–	550	–	1150
Sa_1	5	30	–	–	–	–	–	–	35
Sa_2	–	250	–	–	–	–	226·6	–	516·6
Cg_1	155	390	–	200	–	–	533·3	–	1278·3
P_1	120	1140	–	–	48	–	30	–	1338

bands against the homologous antigen indicating the presence of similar antigenic sites of all these polysaccharides (figure 3). In general, bands 1 and 3 were very thick and 2 and 4 were thin and formed the precipitin lines. In some of the isolates, the precipitin lines 2 and 4 were distinct but in others one or other was merged with the adjacent thick

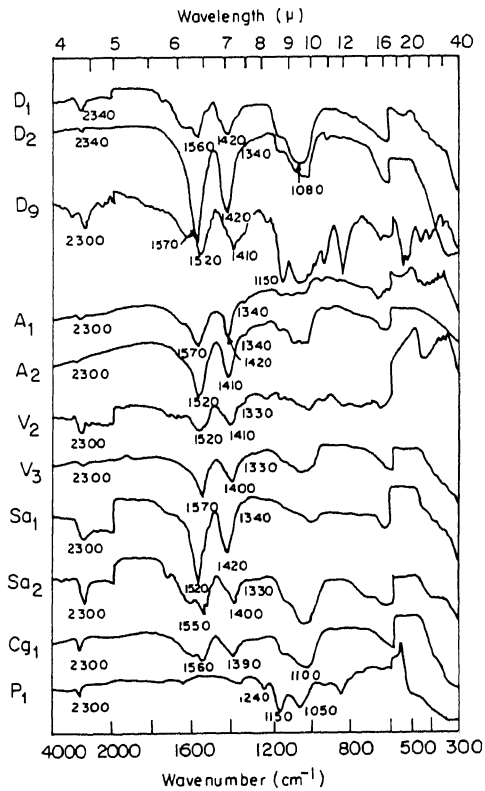


Figure 2. Infrared spectra of the purified extra-cellular polysaccharides of rhizobial isolates.

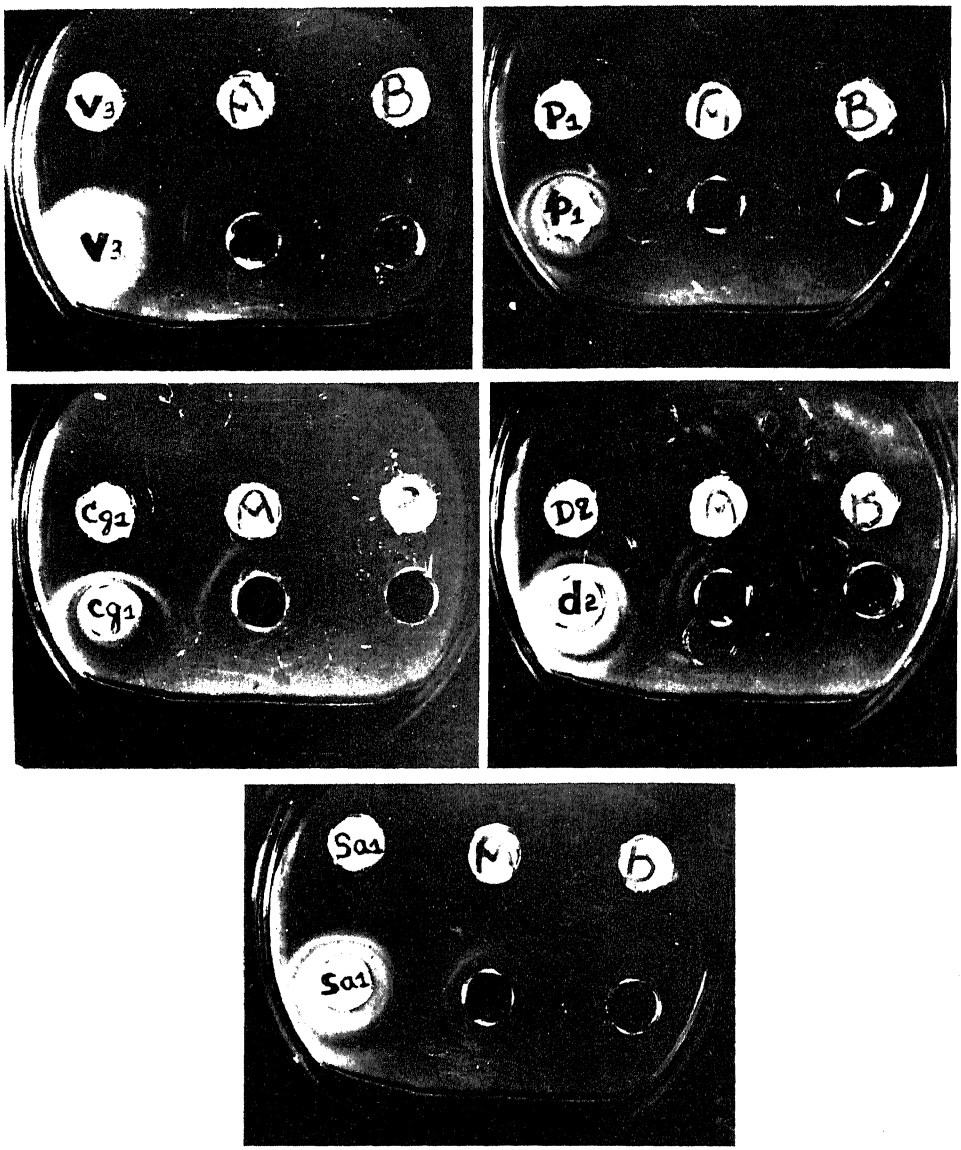


Figure 3. Demonstration of the immunogenicity of the extra-cellular polysaccharides of the different rhizobial isolates (P_1, Sa_1, V_3, d_2 and Cg_1) **A.** Broken cells (Antigen A). **B.** Cell-free extract (Antigen B).

band. With antisera V_2, Sa_2 and P_1, the second precipitin line appeared to merge with band 1. In the isolates D_2, D_9, V_2, V_3, Sa_1 and P_1 there was also an additional hazy line. In Sa_1 and V_3, the fourth line is absent.

Antigen A and B from each isolate when tested along with the polysaccharide antigen against the respective homologous antiserum, do not produce any precipitin lines ruling out the possibility of contamination from cell wall polysaccharides or the cell contents, in the induction of antibodies in rabbits. It is also evident from the above

results that the extra-cellular polysaccharides under study are not analogous with the cell wall polysaccharide.

4. Discussion

It is evident from the results that the purified exopolysaccharides of all the isolates were immunogenic, although only the antigenic nature of the polysaccharide was reported earlier (Dudman 1964). The possibility of contaminating cellular components released by lysis of cells or cell wall lipopolysaccharides in producing the antibodies in rabbits is ruled out as shown by the negative reactions of the antigen A and B (Humphrey and Vincent 1969) (figure 3). It is convincing from the above results that the purified exopolysaccharide used in immunizing the rabbits were not analogous with any of the above mentioned antigens.

In spite of the wide variation in the component sugars which constitute the specificity of the antigenic sites (Wilkinson *et al* 1954; Wilkinson 1958), the polysaccharide of all the isolates showed identical antigenic sites as shown by the similar immunodiffusion reaction. The same type of serological reactions found with the polysacchride antigens could be also due to the presence of the identical functional groups. The occurrence of similar spectra in the longer wavelengths of IR, inspite of the dissimilarities in the shorter wavelengths reflects the inherent structural similarities of these exopolysaccharide.

There was dissimilarity only in the functional group of P_1 isolate. However, this was not reflected in the immunological reactions. The differences in the spectra of the isolates D_9, A_1 and V_2 beyond 8·5 μ might indicate their chemical dissimilarity rather than their functional dissimilarity. Eventhough, the differences in the chemical constitution is responsible for the immunological specificity, serological diversity of chemically similar substances and similarity of quite different polysaccharides were reported (Landsteiner 1962).

The presence of uronic acids as reported in the exopolysaccharide of the isolates D_1, A_1 and Cg_1 does not contribute to chemical differences and the cross reactions depend upon the presence of related or identical constituents.

In conclusion, it may be stated that the rhizobial polysaccharides are very different from those of most of the bacteria where the bacterial slime is made of acid polysaccharides or acid mucopolysaccharides (Clausen 1969). These acidic polysaccharides are not antigenic as is shown in the case of capsules of *Escherichia coli* (Orskov *et al* 1963). In some bacteria, the polysaccharides show much a highly specific reaction that it is possible to use the antigen-antibody reaction for identification of carbohydrates and glycosidic bonds in different carbohydrate antigens (Clausen 1969). Thus the presence of glucose and galactose as monomers, amide and carboxyl as functional groups and the formation of the four characteristic precipitin bands with homologous antisera might well be distinctive characteristics of the polysaccharides of rhizobia. These could possibly be utilised in conjunction with other cultural, physiological and biochemical characteristics to identify rhizobial isolates.

Acknowledgement

One of the authors (RKB) is grateful to UGC for the fellowship and to the Director, University of Madras for providing necessary facilities. Special thanks are due to Dr R

Balasubramanian Lecturer, CAS in Botany and Dr V G Malathi, Scientist, CTCRI, Trivandrum. This paper is part of the Ph.D. thesis approved by the University of Madras.

References

Bailey R W, Greenwood R M and Graig A 1971 Extracellular polysaccharides of *Rhizobium* strains associated with Lotus species; *J. Gen. Microbiol.* **65** 315–322

Block R J, Durram B L and Zweig G 1963 *A manual of paper chromatography and paper electrophoresis* (New York: Academic Press)

Clausen J 1969 *Immunochemical techniques for the identification and estimation of macromolecules.* (Amsterdam, London: North Holland) pp. 571

Dudman W F 1964 Immune diffusion analysis of the extracellular soluble antigens of two strains of *Rhizobium meliloti; J. Bacteriol.* **88** 782–794

Goulden J D S and Sharpe M E 1958 The infrared absorption of Lactobacilli; *J. Gen. Microbiol.* **19** 76

Heidelberger and Avery 1962 in *The Specificity of serological reactions* by Karl Landsteiner (New York: Dover Publications Inc.) 218–219

Hepper M C 1972 Composition of extra cellular polysaccharides of *Rhizobium trifolii; Antonie van Leeuwenhoek* **38** 437–445

Humphrey B A and Vincent J M 1959 Extra-cellular polysaccharides of rhizobium; *J. Gen. Microbiol.* **21** 477–484

Humphrey B A and Vincent J M 1965 The effect of calcium nutrition on the production of diffusible antigens by *Rhizobium trifolii; J. Gen. Microbiol.* **41** 109–118

Humphrey B A and Vincent J M 1969 The somatic antigens of two strains of *Rhizobium trifolii; J. Gen. Microbiol.* **59** 411–425

Landsteiner K 1962 *The specificity of serological reactions* (New York: Dover Publications Inc.)

Levine S, Stevenson H J R and Kablar P W 1953 Quantitative studies of Pneumococcal polysaccharides by infrared spectrophotometry; *Arch. Biochem. Biophys.* **45** 65

Liu P V 1961 Identification of pathogenic *Pseudomonas* by extra cellular antigens: *J. Bacteriol.* **81** 28–30

Orskov I, Orskov F, Jann B and Jann K 1963 Acidic polysaccharide antigen of a new type from *E. coli* capsules; *Nature (London)* **200** 144–146

Sevag M G, Lackman D B and Smolens J 1938 The isolation of the components of streptococcal nucleoproteins in serologically active form; *J. Biol. Chem.* **124** 425–436

Sutton J V and Williams P G 1970 Comparison of extracellular polysaccharide of *Xanthomonas campestris* from culture and from infected cabage leaves: *Can. J. Bot.* **48** 645–651

Vincent J M 1977 Rhizobium—general microbiology; In *A treaties in dinitrogen fixation Section III Biology* (eds) R W F Hardy and W S Silver (New York, London: John Wiley) pp. 227–367

Wagner G H, Cooper F P and Bishop C T 1973 Extra cellular carbohydrate antigens from some non-pathogenic *Neisseria* spp; *Can. J. Microbiol.* **19** 703–708

Wilkinson J F, Duguid J F and Edmunds P N 1954 The distribution of polysaccharide production in *Aerobacter* and *Escherichia* strains and its relation to antigenic character; *J. Gen. Microbiol.* **11** 59–72

Wilkinson J F 1958 The extra-cellular polysaccharides of bacteria; *Bact. Rev.* **22** 46–73

Zevenhuizen L P T M 1971 Chemical composition of exopolysaccharides of *Rhizobium* and *Agrobacterium; J. Gen. Microbiol.* **68** 239–243

Zweig G and Whitaker J R 1971 *Paper chromatography* and electrophoresis (New York, London: Academic Press) Vol 2 pp. 152–169

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 583-587
© Printed in India.

Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India)

G R HEGDE

Department of Botany, Karnatak University, Dharwad 580 003, India

MS received 11 October 1983; revised 2 April 1985

Abstract. Hydrobiological studies of two ponds and two lakes of Dharwad (Karnataka State) for one year showed highest biomass in the nutrient rich Laxman Singh pond. Devaragudihal lake was comparatively poor in nutrients and showed lowest phytoplankton biomass. Both Kyarakoppa pond and Nuggikeri lake did not exhibit significant difference with respect to physical and chemical factors and also standing crop of phytoplankton.

Keywords. Phytoplankton biomass; ponds; lakes; hydrobiological studies.

1. Introduction

Ecological studies carried out in Karnataka for the last decade include physical and chemical measurements and phytoplankton estimations of freshwater habitats. Hosmani and Bharati (1980a, b) studied comparative phytoplankton ecology of four water bodies of Dharwad and algal indicators of organic pollution. Causative factors for certain algal blooms are given by Hosmani and Bharati (1975) and Somashekhar and Ramaswamy (1982).

The present paper is based on a one year (February 1976–January 1977) hydrobiological study of two ponds (Laxman Singh pond and Kyarakoppa pond) and two lakes (Nuggikeri lake and Devaragudihal lake) situated in Dharwad (15° 25′ N lat; 75° E long). The maximum and minimum temperature recorded are 38 and 12° C and the average rainfall is 380 mm. Laxman Singh pond is contaminated by sewage and was devoid of macrovegetation. As Devaragudihal lake continuously supplied water to the Hubli Railway Department; fishing, washing clothes and cattle-bathing were prohibited in this lake. Both Kyarakoppa pond and Nuggikeri lake were disturbed by cattle-bathing, washing of clothes and occasional duck hunters. These water bodies supported macrophytes including species of *Hydrilla, Vallisneria, Najas* and *Nymphaea.*

2. Material and Methods

Surface water samples from five sampling sites in each water body were collected once a month to study the chemical factors. Temperature and pH were measured in the field. Other factors (Oxygen, carbondioxide, carbonates, bicarbonate, major cations, chloride, nitrate, phosphate, silica, iron, oxidizable organic matter and free ammonia) were analysed in the laboratory, transferring the samples immediately, as prescribed by APHA (1965). Simultaneously, separate 500 ml samples were collected, from each water

body, for the estimation of phytoplankton chlorophyll a. Pigment extract was made with 90 % acetone and measured with Spectronic-20 at wavelengths of 630μ, 645μ and 663μ (Happey-Wood 1975).

Table 1. Average values of physical and chemical factors in ponds and lakes of Dharwad (February 1976–January 1977).

Factors	Laxman Singh pond (1)	Kyarakoppa pond (2)	Nuggikeri lake (3)	Devaragudi-hal lake (4)
Temperature water (°C)	24·4	22·8	22·3	25·0
pH	9·1	8·7	8·8	9·2
Dissolved oxygen (ppm)	9·7	12·6	7·5	14·6
Free carbondioxide (ppm)	50·9	41·5	29·9	3·7
Carbonates (ppm)	0·4	0·2	0·2	0·5
Bicarbonates (ppm)	11·4	6·7	9·1	2·8
Calcium (ppm)	39·0	21·5	19·1	14·1
Magnesium (ppm)	4·9	6·6	5·0	3·0
Sodium (ppm)	206·5	153·4	154·4	79·6
Potassium (ppm)	85·8	23·3	20·8	4·8
Chloride (ppm)	122·5	83·8	65·9	32·3
Nitrate (ppm)	4·0	0·8	1·3	0·2
Phosphate (ppm)	0·05	0·01	0·02	0·01
Silica (ppm)	10·5	6·1	5·4	6·2
Iron (ppm)	0·6	1·4	0·7	0·4
Oxidizable organic matter (ppm)	8·7	6·9	7·0	3·0
Free ammonia (ppm)	0·5	0·2	0·2	0·1

Calculated values of $t_{(1,2)} = 2{\cdot}210$; $t_{(1,3)} = 2{\cdot}551$, $t_{(1,4)} = 2{\cdot}272$, $t_{(2,3)} = 1{\cdot}86$, $t_{(2,4)} = 2{\cdot}176$, $t_{(3,4)} = 1{\cdot}946$.

Table 2. Phytoplankton biomass (mg/m^3) in ponds and lakes of Dharwad (February 1976–January 1977).

Months	Laxman Singh pond (1)	Kyarakoppa pond (2)	Nuggikeri lake (3)	Devaragudihal lake (4)
Feb 76	47·5	1·5	3·6	1·0
Mar 76	48·8	4·0	5·8	1·7
Apr 76	123·2	16·0	3·2	1·7
May 76	171·2	23·4	3·3	2·5
Jun 76	19·2	35·7	2·9	1·5
Jul 76	17·8	24·0	4·3	4·2
Aug 76	10·4	5·1	3·3	3·6
Sep 76	4·3	3·6	6·6	4·3
Oct 76	10·4	6·2	8·1	8·1
Nov 76	26·5	10·6	31·0	3·9
Dec 76	35·7	4·4	8·4	3·5
Jan 77	45·5	3·5	1·5	0·7
Average	46·8	11·5	6·8	3·1

Calculated values of $t_{(1,2)} = 2{\cdot}322$, $t_{(1,3)} = 3{\cdot}560$, $t_{(1,4)} = 2{\cdot}966$, $t = 1{\cdot}120$, $t_{(2,4)} = 2.602$, $t_{(3,4)} = 1{\cdot}700$.

3. Results

The average values of various physical and chemical factors analysed are tabulated in table 1. Of the four water bodies Laxman Singh pond showed highest values for almost all the factors with significant differences from the other three water bodies ($p > 0{\cdot}005$). Such a relation between Kyarakoppa pond and Nuggikeri lake did not exhibit significant difference ($p < 0{\cdot}05$), but a significant difference existed between Kyarakoppa pond and Devaragudihal lake ($p > 0{\cdot}005$). Further, the two lakes had almost similar nutrient status.

Phytoplankton biomass, calculated from the estimation of chlorophyll a, is of an order of magnitude greater in Laxman Singh pond (46·8 mg/m^3) than the other three water bodies (table 2), the lowest being in Devaragudihal lake (3·1 mg/m^3). No significant difference in phytoplankton biomass prevailed in the two lakes ($p < 0{\cdot}005$). With regard to phytoplankton biomass, the physical and chemical factors of Kyarakoppa pond was significantly different from that of Devaragudihal lake ($p > 0{\cdot}005$) but with Nuggikeri lake the difference was not significant ($p < 0{\cdot}005$).

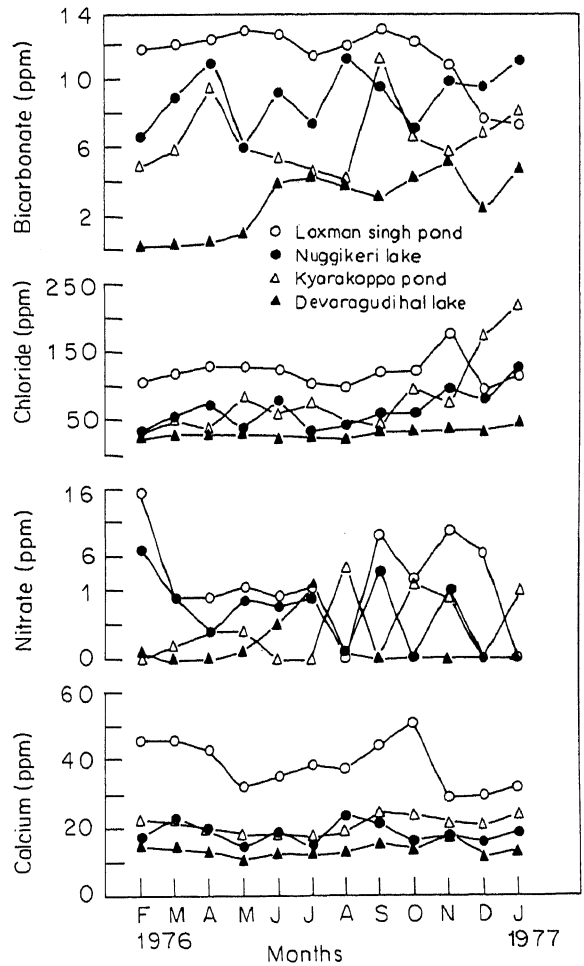
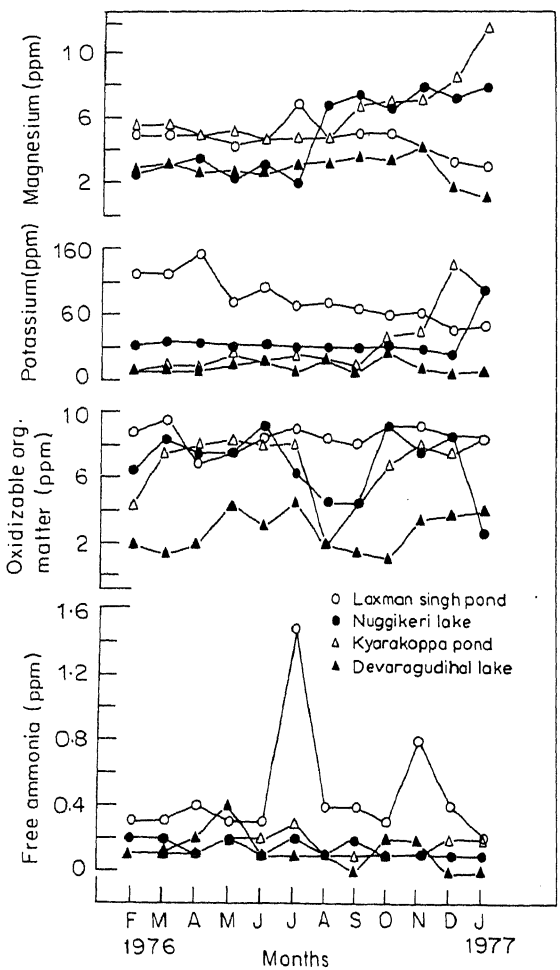


Figure 1.

4. Discussion

Sewage contamination was responsible for the higher nutritional status and phytoplankton biomass in Laxman Singh pond. Devaragudihal lake, undisturbed and uncontaminated, had the lowest nutrient values and also biomass. The relationship of the two lakes with Kyarakoppa pond was quite different. Though the two lakes had similar status with respect to physical and chemical factors and also biomass, Kyarakoppa pond showed significant difference with Devaragudihal lake but not with Nuggikeri lake. From table 1 it can be predicted that factors such as bicarbonate, calcium, magnesium, potassium, chloride, nitrate, dissolved organic matter and free ammonia were probably responsible for such a varied biomass of phytoplankton in these four water bodies. The monthly variations of these factors (figures 1 and 2) also indicate that Devaragudihal lake had the lowest values and Laxman Singh pond the highest values in almost all the months. Further, Kyarakoppa pond and Nuggikeri



Figures 1 and 2. Variations in chemical concentrations in ponds and lakes of Dharwad (February 1976–January 1977).

lake had almost similar values, lesser when compared to Laxman Singh pond and greater than Devaragudihal lake.

It can be concluded, therefore, that diversity in the physical and chemical factors was responsible for the difference in biomass in the four water bodies studied. Laxman Singh pond was richest in nutrients and hence had highest standing crop, whereas, Devaragudihal lake, because of its nutrient poor nature, had less growth of phytoplankton. With respect to both nutrient level and phytoplankton biomass, Nuggikeri lake had a status in between Kyarakoppa pond and Devaragudihal lake. Similar observations have been made by Happey-Wood (1975) for the lakes of North Wales. In general, the present study indicated that the two ponds had more growth of phytoplankton compared to the two lakes, an observation also made by Hosmani and Bharati (1980) for the Dharwad waters.

Acknowledgements

Thanks are due to Prof. S G Bharati for the suggestion and encouragements.

References

American Public Health Association 1965 *Standard methods for the examination of water and waste water, including bottom sediments and sludges* 12th edn (New York: Broadway) 769 pp.

Happey-Wood C M 1975 Distinctions in algal ecology and production in two linked upland lakes, Gwynedd, N. Wales; *Verh. Internat. Verein. Limnol.* **19** 1045–1056

Hosmani S P and Bharati S G 1975 Hydrobiological studies in ponds and lakes of Dharwad. III Occurrence of Euglenoid blooms; *J. Karnatak Univ. Sci.* **20** 151–156.

Hosmani S P and Bharati S G 1980a Limnological studies in ponds and lakes of Dharwar-comparative phytoplankton ecology of four water bodies; *Phykos* **19** 27–43

Hosmani S P and Bharati S G 1980b Algae as indicators of organic pollution; *Phykos* **19** 23–26

Somashekhar R K and Ramaswamy S N 1982 Physico-chemical factors influencing the *Merismopedia* bloom; *Phykos* **21** 62–63

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 589-606
© Printed in India.

Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.)* —I. Stem-bark and root

R C GUPTA
National Botanical Research Institute, Lucknow 226001, India

MS received 6 January 1984; revised 26 December 1984

Abstract. "Dhava" is unanimously ascribed to *Anogeissus latifolia* Bedd. which is a large deciduous tree having most of the curative properties as credited to its stem-bark, root and leaves. A detailed pharmacognosy of the stem-bark and the root of the above species has been dealt with in the present paper. The study revealed that while the stem-bark is characterised by its short-complete fracture, exclusively narrow phloem rays and small irregular concentric phloem fibre strands; the root is identified for its bark having fracture laminated, some considerably broad phloem rays in outer part of phloem, large regular concentric phloem fibre strands and the wood with diffused vessels, xylem parenchyma typically aliform to confluent with some scattered cells and xylem rays exclusively narrow. The fluorescence analysis of drug powders and their behaviour on treatment with different chemical reagents, percentage extractives and ash values, preliminary phytochemical studies including TLC patterns of various extractives have also provided useful parameters to distinguish these plant-parts and check the adulteration.

Keywords. Dhava; *Anogeissus latifolia*; Pharmacognosy; stem-bark and root; Combretaceae.

1. Introduction

"Dhava", which is unanimously attributed to *Anogeissus latifolia* Bedd. syn. *Conocarpus latifolia* Roxb. (Family—Combretaceae) has most of the curative properties as credited to its stem-bark, root and leaves (Anonymous 1948; Basu *et al* 1962; Bhandari 1949; Charak 1949; Dymock *et al* 1891; Ganguli *et al* 1945; Gupta 1956; Kirtikar and Basu 1933; Nadkarni 1954; Mohammed 1970; Sushrut 1954; Vagbhatt 1950; Watt 1889). It is a large or moderate-sized, gregarious tree with an erect, whitish or greyish green trunk. The tree is common throughout India except Assam and ascends to 1350 m in the Himalayas and South Indian hills (Anonymous 1948; Chopra *et al* 1958; Cooke 1901; Gamble 1915; Gupta 1956; Oomachan 1977) and is known by different vernacular names (Kirtikar and Basu 1933).

2. Brief review

Dymock *et al* (1891) have described isolation of 15·5 % of tannic acid from a pale-yellow decoction of its leaves. Recently the leaves are also reported to contain hydrolysable tannins of which gallotannins accounted for 90–92 %. Shikimic acid, dihydroshikimic acid, quinic acid, gallic acid, chebulagic and trigallic acid along with monosaccharides-arabinose, rhamnose, fructose, glucose and galactose were present. The amino acids

* NBRI Research Publication No. 243 (New Series)

alanine, glycine and phenylalanine were identified. A glucoside highly soluble in water and insoluble in chloroform (m.p. 158–59°) has been isolated from an aqueous extract of these (Rao and Sastry 1960; Reddi *et al* 1962, 1965). Joshi (1959) studied the foliar calcium.

The stem-bark contained sitosterol, flavellagic acid 3,3,4-tri-*o*-methyl ellagic acid, quercetin, myrecetin and procynidin along with gallotannins. Shikimic acid, quinic acid and free sugars—glucose and galactose were detected. It also contained alanine and phenyl alanine. The heart-wood has the same components as the bark except that in amino acids only phenylalanine was found (Reddi *et al* 1962a, 1964, 1965a).

The alcoholic extract of the stem-bark was found to have CNS-depresent activity in mice. It produced hypothermia and also responded to amphetamine hyper activity test (Bhakuni *et al* 1971).

There seems to be no account available in the field of pharmacognosy except those of Eldo *et al* (1977) and Tiagi (1969) relating respectively to the occurrence of septate macrosclereids in the stem bark of this species and vascular anatomy of the flowers of the genus *Anogeissus* without taking into consideration the distinguishing pharmacognostical characters of this species. The present work, dealing with a detailed pharmacognostical study on stem-bark and root of *Anogeissus latifolia* was, therefore, undertaken.

3. Material and methods

The present study is based on the materials collected from the Institute campus in September–October. Temporary mount of hand sections were employed for anatomical studies. For the study of isolated cells of different tissue, small pieces of the material were macerated separately in Schultz's fluid. Microchemical test was performed according to Johansen (1940) and Kay (1938). Fluorescence analysis was carried out according to the method described by Kokoski *et al* (1958). Recourse was also taken to study the behaviour of powders on treating with certain chemical reagents. Methods recommended in IP (Anonymous 1966) were followed for determining ash values and percentage extractives. Preliminary phytochemical studies and TLC pattern on silicagel G were studied according to the method of Rosenthalar (1930) and Randerath (1963), respectively.

4. Observation

4.1 *Organoleptic characters*

4.1a *Stem-bark* (*figure* 1A): The pieces of dry bark which vary in size are usually 0·9–1·26 cm thick and externally pale-grey, smooth with small ridges at places. The inner surface being pale-brown and longitudinally striated. The bark fractures sharp and complete and has a slight bitter and astringent taste with a characteristic odour.

4.1b *Root* (*figure* 1B, C): The young roots, less than 2 cm in diameter, have 0·1 to 0·2 cm thick bark with distinct longitudinal wrinkles, scars of root-lets and some scattered oval to round small lenticels. The mature root pieces are stout with light grey to dark greyish brown exterior having a hard and compact rind (rhytidoma),

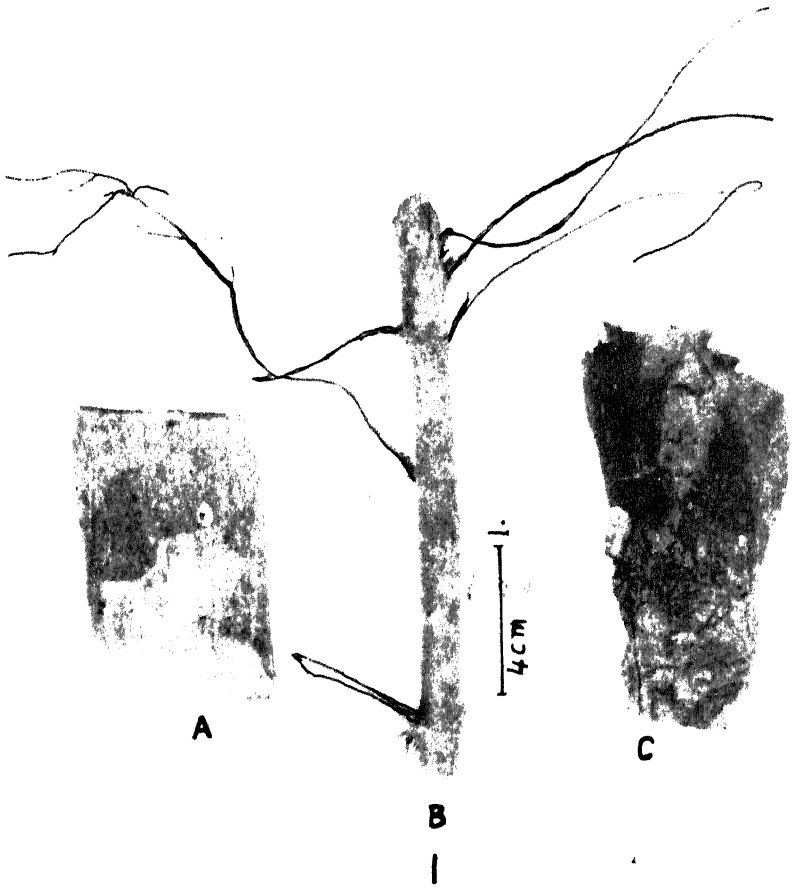
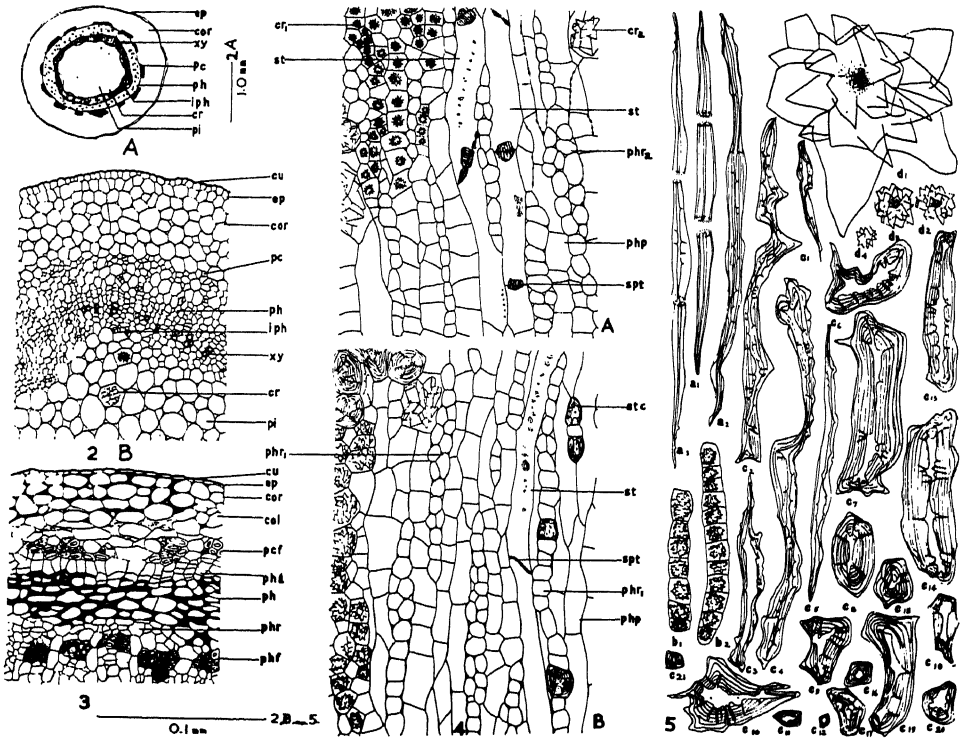


Figure 1. Macroscopic features of stem-bark and root of *Anogeissus latifolia*. **A.** a portion of the stem-bark. **B.** a portion of the root. **C.** a portion of the root-bark.

comprising nearly one-third the thickness of the entire bark, which exfoliates in thin, hard flakes of various shapes and sizes, leaving smooth irregular depressions with a thin soft corky skin on the surface. Roots fracture laminated in bark and fibrous in wood region and have an astringent taste with no characteristic odour.

4.2 *Microscopic characters*

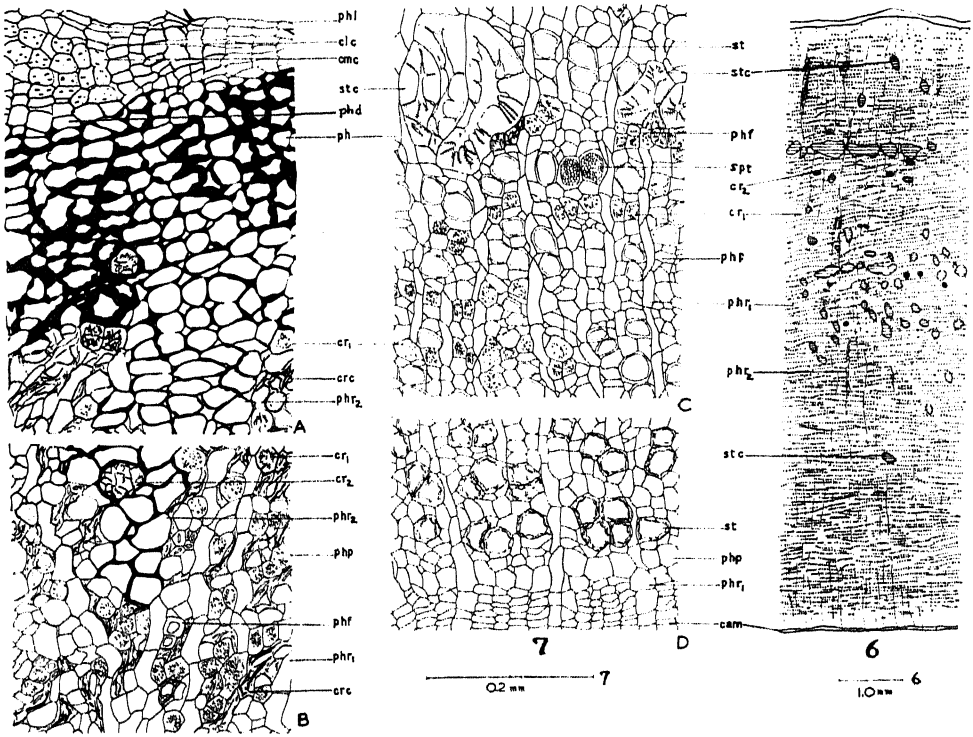
4.2a *Stem-bark* (*figures* 2–7): *The young stem and development of bark*: The young stem shows a layer of epidermis composed of cubical to radially elongated cells (figures 2A, B). The cortex, which consists of 5–7 cells wide parenchyma at the early stage, soon becomes collenchymatous in its outer 3–4 layers and many parenchymatous cells inner to these collapse. Small patches of thin-walled polygonal cells separated by groups of isodiametric parenchymatous cells compose the pericycle which surrounds the amphephloic siphono-stele (figures 2A, B). The endodermis is not distinct at this stage, but soon gets demarcated as a distinct layer of starch-sheath above the pericycle. The



Figures 2–5. Microscopic characters of stem-bark of *Anogeissus latifolia*. **2A.** Diagrammatic TS of young stem. **B.** Cellular details of a portion of figure 2A. **3.** Details in TS of a slightly mature stem showing formation of phellogen in the outermost phloem cells. **4.** Parts of tangential section of the bark. **A.** through inner phloem, **B.** through outer phloem. **5.** Isolated elements of bark, **a1–a3.** phloem fibre, **b1–b2.** crystal fibres, **c1–c21.** sclereids, **d1–d3.** sphaerulites (large), **d4.** cluster crystal (small).
(*See p. 68 for explanations of abbreviations*)

cork cambium arises in the peripheral cell layer of the phloem (figure 3). It produces only a few phelloderm layers in the innerside and a comparatively wider phellem on the outerside. In lenticel regions the cork is represented by alternating rows of complementary cells and closing cells. The phloem cells in the outer region, later, become considerably thick-walled and the pericyclic cells develop as fibres (figure 3). Strands of phloem fibres are produced in concentric bands (figure 3). Cluster crystals of calcium oxalate are formed in most of the phloem parenchyma arranged in tangential rows. In the outer and middle phloem regions small islands of stone cells are developed. The epidermis and cortex alongwith the pericyclic fibres get ruptured.

Mature bark: In a cross-section of the bark (1·25 cm thick) the cork is composed of suberised tangentially elongated cells. In the lenticel region the complementary cells measure 12–16–21–25 × 16–21–29–37 μm and the closing cells 12–16–25–29 × 8–16–25–41 μm (figures 6, 7A). The phelloderm is 1–2 layered. The secondary phloem is characterised by the presence of ceratenchyma, small islands of sclereids, fibres, thick-walled sieve-tubes and companion cells besides parenchyma and crystal fibres (figures 4–7). The phloem cells are thick-walled in the outer region (figures 7A, B). The sclereids have very thick striated walls and distinct ramified pit canals (figure 7C). Some of these



Figures 6–7. Microscopic characters of stem-bark of *Anogeissus latifolia*. **6.** Part of diagrammatic TS of bark. **7A–D.** Cellular details of a portion of figure 6.
(*See p. 68 for explanation of abbreviations*)

are more or less isodiametric, rectangular to irregular in shape, the others being long, branched, resembling the phloem fibres and having their lumen occasionally divided into two or three equal or unequal chambers due to formation of septa (figures 5c1–c20). These cells measure 16–66–103–207 × 29–28–287–625 μm. The phloem fibres occur in small concentric strands which are irregular (figures 7B, C). The cells are long, measuring 16–20–25 × 348–537–1107 μm, thick-walled, lignified and having spathulate and tapering extremeties (figures 5a1–a3).

The sieve elements are almost crushed, forming ceratenchyma in the outer phloem region (figures 7A, B). In the lower middle and inner phloem the sieve tubes are functional and measure 20–29–37–49 × 248–310–360–419 μm (figures 7C, D). These are usually thick-walled, having sieve plates both simple and compound on the oblique ends or side walls and show distinct callus plugs sometimes (figures 4A, B). The compound sieve plates have sieve areas arranged in a scalary manner.

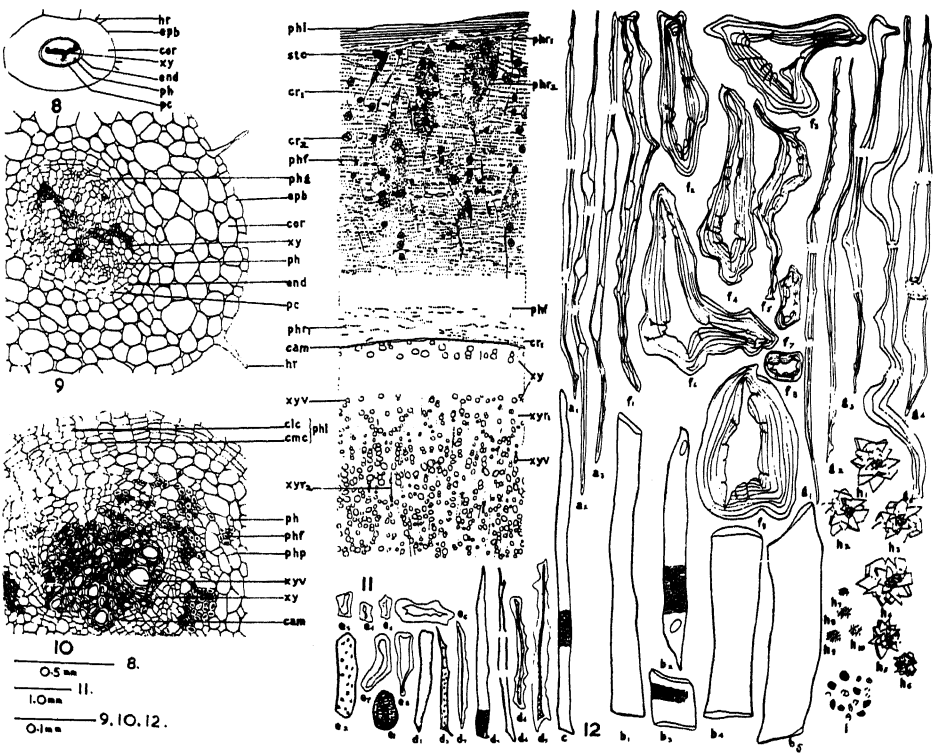
The phloem parenchyma are more or less polyhedral in shape and often contain cluster crystals measuring 12–24–33–45 × 16–25–32–49 μm (figures 5d2–d4, 7). The large, solitary sphaerulites which measure 118–168–213–254 × 116–205–230–283 μm occur less frequently (figures 5d1, 7B).

In tangential sections the uni- and bi-seriate rays are 4–9–14–32 cells high, where as the multi-(tri-) seriate ones are usually 13–23 cells high (4A, B). The ray cells are mostly thin-walled, measure 16–25–29–41 × 10–16–20–31 μm. Some of these, however, become thick-walled and lignified to form stone cells (figure 4B).

Following the phloem is 3–6 layers of cambium of somewhat rectangular cells measuring 8–10–12–16 × 8–16–24–33 μm (7D).

4.2b *Root* (*figures* 8–14): *The young root and development of root bark*: It shows a spherical or oval outline in a cross-section (figure 8). The epiblema is single layered with some root hairs (figures 8, 9). Following this is 4–6 layers of parenchymatous cortex. A well defined endodermis is followed by a layer of thin-walled cells forming the pericycle. The central core of the root is occupied by a tri-arch stele having alternating, radial groups of xylem and phloem (figure 9).

The secondary growth starts in the normal way and the phellogen arises quite early in the pericycle (figure 9). As the cork cells are formed, the cortex gets compressed and finally ruptured away along-with epidermis. The phellem cells are rectangular and flat in the young root but when lenticels cells also develope, several alternating rows of two types of cells are differentiated—one being rectangular, flat and compactly arranged, the closing cells and the other being somewhat spherical and loosely arranged, the complementary cells (figure 10). The phloem produces isolated or group of phloem fibres and the stone cells (figure 10). Clusters of calcium oxalate crystals are deposited



Figures 8–12. Microscopic characters of root of *Anogeissus latifolia*. **8.** Diagrammatic TS of root. **9.** Part of TS showing cellular details of figure 8. **10.** Part of TS of slightly mature root showing fibre formation in phloem. **11.** Part of diagrammatic TS of mature root. **12.** Isolated elements of root: **a1–a3.** libriform xylem fibres, **b1–b5.** vessel elements. **c.** a tracheid fibre; **d1–d7.** tracheids; **e1–e8.** xylem parenchyma; **f1–f9.** sclereids; **g1–g5.** phloem fibres; **h1–h6.** sphaerulites; **h7–h10.** cluster crystals; **i.** starch grains.

(*See p. 68 for explanations of abbreviations*)

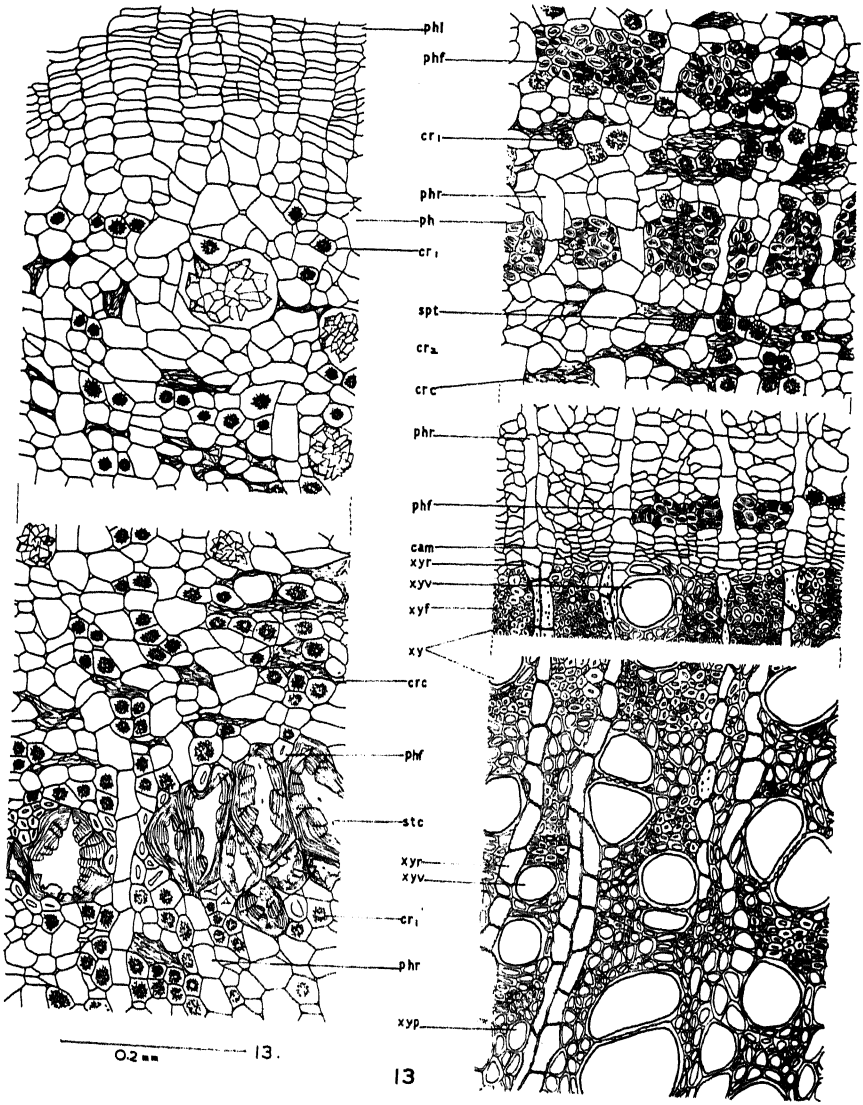


Figure 13. Microscopic characters of root of *Anogeissus latifolia*. Cellular details of a portion of figure 11.
(*See p. 68 for explanations of abbreviations*)

in the phloem parenchyma. The patches of cork cambium develop at various places in the phelloderm and the phloem also.

Mature root: In about 2 cm thick root the cork is composed of 7–16 or more layers of tangentially elongated suberised cells measuring 16–20–28–37 × 4–8–10–16 μm. The phellogen is distinct and the phelloderm is formed of 2–5 layers of parenchymatous cells measuring 20–32–40–61 × 20–24–28–36 μm. Most of the cells of the latter are tanniniferous and also contain clusters of calcium oxalate crystals.

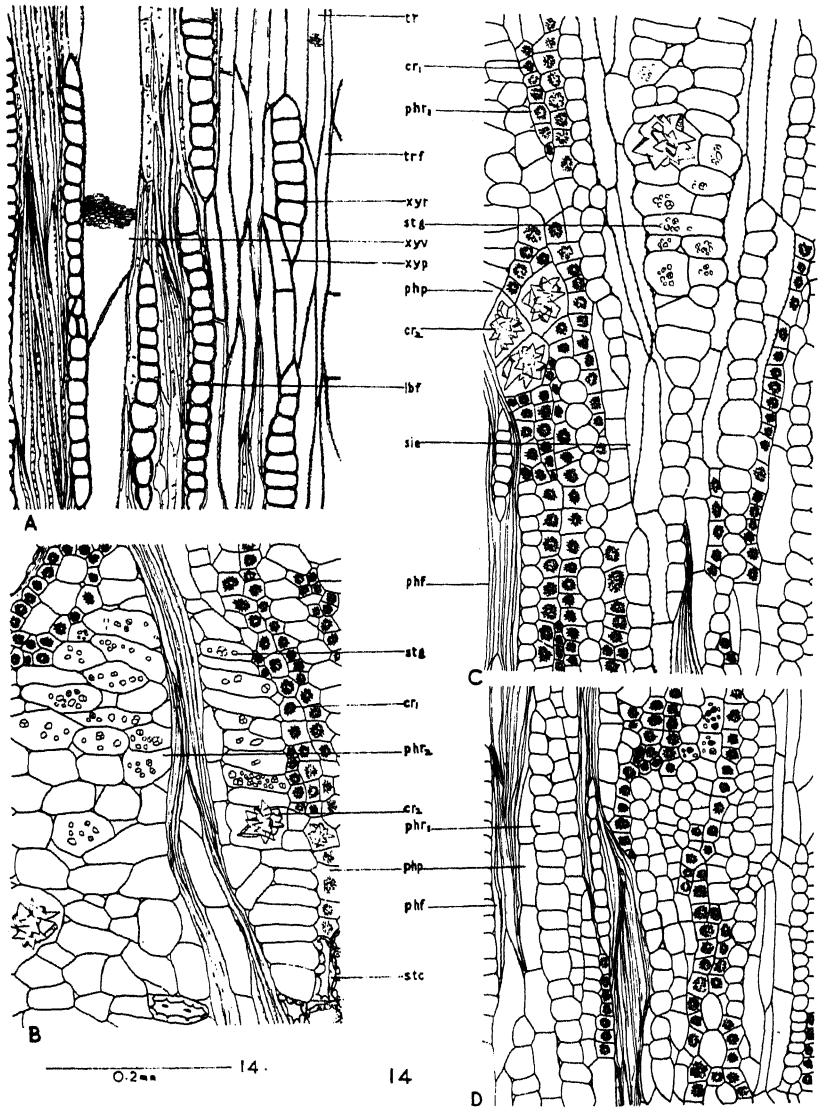


Figure 14. Microscopic characters of root of *Anogeissus latifolia*. Part of tangential sections of root. **A.** through xylem; **B.** through outer phloem; **C.** through middle phloem; **D.** through inner phloem.
(*See p. 68 for explanation of abbreviations*)

In much older roots, however, the outer bark consists of rhytidoma composed of phellem layers alternating with phelloderm and phloem tissue and being more or less crushed.

After the removal of rhytidoma the remaining portion of the bark consists of a few layers of periderm (only phellem cells) and a broad secondary phloem composed of phloem parenchyma, sieve elements, ceratenchyma, sclereids, phloem fibres and rays (figures 11, 13). Calcium oxalate crystals occur in abundance throughout the phloem

region (figure 13). Of the two types of crystals, the large sphaerulites measuring 66–115–164–230 × 66–98–181–213 μm occur scattered in some of the phloem parenchyma (figure 12h1–h6, 13), whereas as the small cluster crystals of calcium oxalate measuring 16–33–49 × 16–24–41–49 μm are found in phloem parenchyma which are arranged in tangential rows alternating with phloem fibres (figures 11, 12h7–h10, 13). The sclereids occur as small islands in the outer parts of the phloem (figure 13). These vary in shape and size and measure 41–164–395–576 × 8–49–98–279 μm (figure 12f1–f9). The phloem fibres are usually found in large tangential strands arranged in regular concentric bands (figures 11, 13) which lignified and measure 658–1266–1809–2352 μm in length, having 3–16–24–33 μm diameter at their widest part. Their lumen is very narrow and extremeties truncated and tapering (figure 12g1–g5). The sieve tubes and companion cells are usually collapsed and form the ceratenchyma. The sieve elements are functional in the inner phloem (figure 13). The sieve tubes have simple sieve plates at their oblique ends and sometimes show distinct callus plugs, as in the stem-bark (figure 14C). These measure 264–333–404 μm in length and 33–42–46 μm in breadth. The phloem parenchyma contain tannins. The phloem rays are uni-, bi- and multiseriate (3–8 or more cells wide). The uni- and bi-seriate rays are 12–35–40–65 cells high and the multiseriate ones 14–25–35–50 cells high (figure 14B–D). The individual ray cells of uni- and bi-seriate rays measure 16–49–65–98 × 16–41–49–65 μm and the multiseriate ray cells 24–65–115–180 × 41–65–99–180 μm.

Following the phloem is a 2–4 layered-cambium having thin-walled, more or less rectangular cells (figures 11, 13).

The secondary xylem composed of the usual cells is diffused porous with inconspicuous growth rings (figures 11, 13). The vessel elements are moderately numerous (count varies between 35–45 mm^2), mostly solitary and in short radial multiples of 2–3. Also there is some tendency towards tangential or oblique groupings in these (figures 11, 13). The cells are long, cylindrical to small drum-shaped (measure 33–82–115–164 × 115–247–362–510 μm) and have alternately arranged bordered pits on their walls (figure 12b1–b5). The tracheids are narrow with tapering and truncated extremeties (figure 12d1–d7). The pits on their walls are both alternately arranged distinctly bordered type (figure 12d1–d3) and reduced pits as to appear simple slit like (figure 12d4–d7). Those tracheids which have numerous bordered pits measure 16–24–33 × 197–312–557–608 μm. The xylem parenchyma are paratracheal, arranged in thin sheaths or eyelets round the vessels, also being confluent, connecting tangentially or obliquely arranged vessels (figure 13). The cells are slightly elongated more or less rectangular in shape, having the usual simple or bordered pits on their walls (figure 12e1–e2). Some of these, however, show very thick walls and no pits (figure 12e3–e8). The xylem fibres are long with tapering ends and the lumen almost obliterated excepting in a few cases, where it is very wide (figure 12a1–a3). The tracheid fibres are exceptionally very long with a structure similar to that in bordered pitted tracheids (figure 12c). The xylem rays are homogeneous exclusively uniseriate and 7–15–22–28 cells high (figure 14A). The cells are radially elongated and measure 50–65–83 × 22–32–42 μm.

4.3 *Cell-contents*

Besides calcium oxalate crystals and dark brown tannins in tanniniferous cells, also the starch as simple and compound granules occurs in stem-bark and root (figure 12i,

14b–d). The simple grains are polyhedral or spheroidal with the hilum sometimes visible as a central point or a small cleft. The compound grains have 2–6 (usually 3–4) components which measure 2–3–8–10 × 2–4–8 μm. These occur in phloem rays and many phloem parenchyma. The xylem rays in the root also contain them abundantly. Besides, steroids, triterpenes, reducing sugars, polysaccharides/glycosides and flavonoids are present in various extractives. The plant bases, coumarins and resins were not traceable (table 4).

4.4 *Examination of drug powders*

While the stem-bark has a greyish brown colour, the powdered root is pale-brown. Both of these have a slight acrid-bitter and astringent taste.

The histological elements of the stem-bark observed microscopically include a large number of stone cells, fragments of phloem fibres and crystal fibres either isolated or in groups, some cork cells and parenchyma having both small and giant clusters of calcium oxalate and starch grains in some of them. Crystals and starch grains occur in isolated form also. The powdered root, besides the above elements, comprises of isolated and small groups of xylem parenchyma, broken xylem fibres, tracheids and vessels.

A small quantity of each of the two drug powders treated separately with different chemical reagents and exposed to UV light emits fluorescence as shown in table 1, behaviour of these powders treated with various chemical reagents is noted in table 2, and the extractive percentage and ash-values, recorded in table 3 are also helpful in identifying each of these. Using TLC pattern of the various extractives the respective powdered parts are easily distinguishable (table 5).

Table 1. Fluorescence characteristics of powdered stem-bark and root of *Anogeissus latifolia.*

Treatment	Fluorescence	
	Stem-bark	Root
Drug powder mounted in nitrocellulose dissolved in amyl acetate.	Yellowish brown	Greyish brown
Drug powder treated with 1N.NaOH in methanol	Blackish brown	Pinkish brown
Drug powder treated with 1N.NaOH in methanol and mounted in nitrocellulose in amyl acetate	Blackish brown	Greyish brown
Drug powder treated with 1N.NaOH (aq.)	Olive-brown	Greenish brown
Drug powder treated with 1N.NaOH (aq.) mounted in nitrocellulose in amyl acetate	Olive-green with violet tinge	Greenish-violet
Drug powder treated with HCl	Brown with violet tinge	Reddish brown
Drug powder treated with 1N.HCl and mounted in nitrocellulose in amylacetate	Yellowish brown	Brown with violet tinge
Drug powder treated with 50% nitric acid (sp. gr. 1·42)	Yellowish green with orange tinge	Green with orange tinge
Drug powder treated with H_2SO_4 (sp. gr. 1·84)	Greenish brown	Blackish green
Drug powder as such	Violet-brown	Violet-brown

Table 2. Effect of different chemical reagents on powdered stem-bark and root of *Anogeissus latifolia.*

Treatment	Colour	
	Stem-bark	Root
Powder + Iodine soln.	Brown	Bluish brown
Powder + $FeCl_3$ soln. (5%)	Bluish green	Bluish green
Powder + Ammonia soln.	Light brown	Dark brown
Powder + Ammonia soln. + Pot. ferricyanide	Turned violet to brown	Turned violet to reddish brown
Powder + 1N.NaOH soln.	Blackish brown	Turned brown
$\frac{\text{Powder}}{\text{extract}}$ + 4% NaOH + 1% $CuSO_4$ soln. heated	Dark tan brown	Turned brown
$\frac{\text{Powder}}{\text{extract}}$ + Sod. nitroprusside soln. + 40% NaOH soln.	Bright tan brown	Bright tan Brown
$\frac{\text{Powder}}{\text{extract}}$ + 40% NaOH soln. + 1% Lead acetate soln. heated	Dark greyish brown	Buff-brown
Powder + Millon's reagent	Brick red	Turned brown to reddish brown
Powder + HNO_3 50% aq. + Ammonia soln.	Orange	Brick red
Powder + Picric acid (Saturated aq. soln.)	Yellow	Dirty yellow
Powder + Lactic acid	Pinkish brown	Light brown
Powder + Acetic acid	Pinkish brown	Light brown
Powder + H_2SO_4 50% soln.	Blackish brown	Brown
$\frac{\text{Powder}}{\text{extract}}$ + Acetic acid + H_2SO_4	Reddish brown	Brown
Powder + HCl conc.	Light pinkish brown	Dull brown
$\frac{\text{Powder}}{\text{extract}}$ + Phloroglucinol + HCl conc. heated	Reddish orange	Orange
$\frac{\text{Powder}}{\text{extract}}$ + Resorcinol + HCl conc. heated	Orange	Orange
Powder + sudan III soln. washed with 50% alcohol and observed under microscope	No change	No change

Table 3. Determination of ash-values and extractive-percentage of *Anogeissus latifolia.*

Plant part	Values			
	Total ash (%)	Acid insoluble ash (%)	Water soluble extractive (%)	Alcohol soluble extractive (%)
Stem-bark	9·195	0·0916	20·8726	18·394
Root	5·98	0·0866	11·371	10·116

Table 4. Percentage—yield, consistency and physical appearance of extractives and their broad chemical constituents.

					Fluorescence under ultra-violet light						Chemical constituents									
Extractives	Plant-parts	Average percentage (w/w)	Nature of residue	Colour of extract	Extractives as such	Extractive treated with nitrocellulose	Extractive treated with 1N. NaOH meth:	Extractive treated with 1N. NaOH meth. and mounted in nitro-cellulose	Extractive treated with 1N. HCl	Extractive treated with 1N. HCl and mounted in nitro-cellulose	*Sterols* / Triterpenes	Alkaloids	Tannins	Reducing sugars	*Polysaccharides* / Glycosides	Saponins	Flavonoids	Resins	Coumarins
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Petro. ether (60–80°)	Stem-bark	0·272	Waxy	Light pale-grey	Spot fluorescence pinkish brown	Bright pink	Purple-violet	Dirty pink	Purple-violet	Bright pink	+	–	–	–	–	–	–	–	–
	Root	0·308	Waxy	Pale-brown	Spot fluorescence cannary yellow	Yellow	Yellow	Bright pale-yellow	Light pale-yellow	Light Yellow	+	–	–	–	–	–	–	–	–
Benzene	Stem-bark	0·096	Waxy	Light pale-grey	Spot fluorescence purple violet	Pink	Purple	Pinkish violet	Purple	Bright pink	+	–	–	–	–	–	–	–	–

	Root	0·114	Waxy	Light olive-brown	Spot fluorescence yellow	Pinkish yellow	Pale-yellow	Light buff-yellow	Bright pale-yellow	Pinkish yellow	+	−	−	−	−	−	−	−	−
Chloroform	Stem-bark	0·188	Sticky solid	Pale-brown	Spot fluorescence pinkish brown	Bright pink	Pinkish brown	Pinkish brown	Violet	Pinkish orange	−	−	+	+	+	−	−	−	−
	Root	0·283	Sticky solid	Yellowish brown	Spot fluorescence pale-yellow	Pinkish pale	Bright pale	Bright pale-yellow	Yellow	Pinkish pale	−	−	+	+	+	−	−	−	−
Alcohol	Stem-bark	19·822	Dry gummy solid	Brown	Spot fluorescence brownish yellow	Brownish yellow	Yellowish brown	Yellowish brown	Pale-brown	Pale-brown	−	−	+	+	+	−	+	−	−
	Root	13·934	Dry gummy solid	Yellowish brown	Spot fluorescence light pinkish brown	Light pinkish brown	Yellowish brown	Dirty yellow	Pale-brown	Pale-brown	−	−	+	−	+	−	−	−	−
Water	Stem-bark	4·668	Dry gummy solid	Tan brown	Spot fluorescence light pale-brown	Light pale-brown	Light pale-brown	Light pale-brown	Light pale-brown	Light pale-brown	−	−	+	−	+	−	+	−	−
	Root	8·028	Amor-phous solid	Yellowish brown	Spot fluorescence brown	Brown	Brown	Brown	Brown	Brown	−	−	+	−	+	−	−	−	−

Table 5. TLC pattern of stem-bark and root of *Anogeissus latifolia* under UV light.

Extractive	Solvent system	Plant-part	No. of spots	hRf value				
1	2	3	4	5				
Petro. ether (60–80°)	Petro.ether-Acetone-chloroform 32:6:2	Stem-bark	12	0·8(Mi) pinkish red	19·2(Ma) pinkish red and long-trailing	39·8(Ma) pink	48·3(Ma) pink	53·8(Mi) pink
				58·4(Mi) pink	64·4(Mi) violet	72·0(Mi) pink	81·3(Mi) bluish green	81·7(T) pink
				94·9*(T) dull white	97·4*(T) dull white			
		Root	17	0·8(T) pink	2·5(T) light pink	6·7(Mi) light bluish green	22·8(Mi) light pink, a trailing	34·7(T) light pink
				38·1*(T) dull white	40·6*(T) dull white	45·7(Mi) pink	53·3*(Mi) dull white	61·0(Mi) dull white
				66·1(Mi) dull white	70·3(Mi) dull white	77·1(Mi) bluish green	82·2*(T) dull white	90·6(T) dull white
				95·7*(T) dull white	97·4(T) dull white			
Benzene	-do-	Stem-bark	6	0·8(T) pinkish violet	16·0(Ma) pink a trailing	33·0(Mi) pink	42·8(Mi) pink	
				95·5(T) violet	97·4(T) white			
		Root	7	0·8(Mi) violet	6·2(Mi) light bluish green	14·2*(Ma) light bluish green	19·2*(T) light pink	
				42·8(T) light pink	95·7*(T) dull white	97·4*(T) dull white		
Chloroform	Benzene-Chloroform-Methanol-Water 28:7:3:2	Stem-bark	8	0·9(T) pinkish violet	3·7(T) light pink	16·8(Mi) dull white	31·7(Mi) dull white	

				41·1(T) dull white	73·8*(Mi) pink	91·5(Mi) pink	98·1(T) dull white	
		Root	8	0·9(Mi) violet	7·4(T) light pink	16·8(Mi) sky-blue	36·4(Ma) light-sky-blue	
				45·7(Mi) light sky-blue	72·8*(T)	92·5*(T)	97·1*(Mi)	
ol	(A) -do-	Stem-bark	3	0·9(Mi) violet	16·8(Mi) dull white	97·1(Mi) dull white		
		Root	5	0·9(Mi) blue	16·8(Mi) light bluish white	26·1(Ma) light bluish white	72·8*(Mi)	
				97·1(Mi) light bluish white				
	(B) Chloroform-Methanol-*n*-propanol-water-dimethylamine 48:6:2:2:2	Stem-bark	6	0·9(Mi) violet blue	7·4(Ma) violet	17·7(Ma) dull white	36·4(Mi) dull white	43·9(T) dull white
				99·0(T) dull white				
		Root	8	0·9(Mi) violet	6·5(Mi) bluish white	11·3(Mi) dull white	16·8(Ma) dull white	
				26·1(Ma) dull white	35·5(Ma) dull white	44·8(Ma) dull white	99·0(Mi) dull white	
	-do-	Stem-bark	4	0·9(Mi) violet blue	3·7(Mi) light violet	37·7*(T) dull white	98·1(Mi) dull white	
		Root	9	0·9(Mi) violet	6·6(Mi) violet	13·0(Mi) dull white	17·9(Mi) dull white	
				30·1(Mi) dull white	38·6(Ma) dull white	48·1(Ma) dull white	55·6(T) white	98·1(Mi) white

n trace; Mi = minor component; Ma = Major component.

4.5 *Phytochemical examination*

Three samples of (20 g) powdered stem bark and root were extracted separately with petroleum ether (60–80°), benzene, chloroform, alcohol and water successively in a Soxhlet extraction apparatus. Fluorescence characteristics of the extractives under UV light were studied by spotting the extract on a non-fluorescent filter paper and treating with various reagents. Solvents from the respective extracts were evaporated and the average yield percentage of each extract calculated. Physical appearance and consistency of the drug extract was noted (table 4). These were further screened for steroids/triterpenes, alkaloids, flavonoids, reducing sugars, saponins, polysaccharides/glycosides, tannins, resins coumarins. The results obtained are given in table 4.

As an additional parameter for characterising the drug a comparative study of the various extractives was also carried out through uni-directional thin-layer chromatography on silicagel G plates. Chromatogram of the respective extractive was exposed to UV light to record the colour fluoresced by different components and their hRf (table 5). The spots, which were not clear in UV light were confirmed by spraying 1 % sulphuric acid in alcohol as detecting reagent followed by heating at 110°C for 30 min and are indicated in the table by asterisks. The results revealed that while petroleum ether extractives of both stem-bark and root respectively gave as many as 12 and 17 UV fluorescent spots including a few trailing in developing solvent system petroleum ether-acetone-chloroform (32:6:2), the benzene extracts of above plant-parts exhibited respectively 6 and 7 spots including a few trailings. Similarly, when a mixture of benzene-chloroform-methanol-water (28:7:3:2) was used as the developing solvent system for chloroform and alcohol extractives of both stem-bark and root the former extracts of each resolved into 8 spots and the latter into 3 and 5 respectively. A more specific TLC of alcohol extractives of stem-bark and root, however, showed respectively 6 and 8 spots in a mixture developing solvent of chloroform-methanol-*n*-propanol-water-diethylamine (48:6:2:2:2) whereas the water extractives respectively gave 4 and 9 spots.

5. Discussion and conclusions

In the present study the primary structure of the stem and root and their secondary development, particularly in the bark region has been considered which may help in identifying the barks of different growth stages (from young to mature).

From the foregoing observations it is evident that there is great similarity in macro- and microscopical characters of both the stem and the root barks. However, there are many salient difference by which these two plant-parts can be easily distinguished. Macroscopically, the stem-bark is characterised by its fracture which is short and complete in comparison to the root-bark which has laminated fracture in its outer part and a fibrous on in the inner. The layers of thick-walled phloem cells occurring in the outer stem-bark are absent in the root. Islands of sclereids occur more frequently in the outer and middle portions of the stem-bark whereas these are less frequent in the root-bark and are observed in its outer portion only. The phloem fibres in the stem-bark are distributed in small irregular concentric strands, while in the root-bark these occur more frequently and are comparatively larger, somewhat regular concentric strands. Whereas occurrence of uni- and bi-seriate rays is a characteristic feature of the two

barks, multiseriate rays are also found frequently in the outer portion of the root-bark. Similarly, the presence of a thick-walled sieve-tube is a characteristic feature of the stem-bark and frequent occurrence of large sphaerulites of calcium oxalate of solitary type characterises the root-bark.

The wood portion of the root may easily be identified microscopically as it has diffuse vessels in both solitary and radial groups of 2–3; xylem parenchyma typically aliform to confluent with some scattered cells, occasionally vasicentric and in terminal bands; xylem fibres with small simple pits, medium length and wide lumen at some places; xylem rays homogeneous and usually uni-seriate.

Besides, the recorded behaviour of drug powders on treatment with different chemical reagents (table 2), fluorescence characteristic of powders and extractives (tables 1 and 4), extractive-percentage and ash-values (table 3) and the TLC pattern of extractives (table 5) also provide remarkable diagnostic criteria.

Acknowledgement

The author is extremely grateful to Director, National Botanical Research Institute for facilities. Thanks are also extended to Sri A Jha, R S Ojha, K Kumar and M K Tandon for technical assistance.

References

Anonymous 1948 *Wealth of India Raw materials* (New Delhi: CSIR) Vol 1 pp. 81–82

Anonymous 1966 *Pharmacopoeia of India* (New Delhi: Govt. of India) pp. 947

Basu D K, Mohan Rao V K and Nayar S L 1962 Gum ghatti—A substitute of gum acacia; *Indian J. Pharmacol.* **24** 256

Bhakuni D A, Dhar M L, Dhar M M, Dhawan B M, Gupta B and Srimal R C 1971 Screening of Indian plants for biological activity, Part III; *Indian J. Exp. Biol.* **9** 91

Bhandari C R 1949 *Vananshadhi Chandrodya* 2nd edn (Bhanupura–Indore: Gyan Mandir) Vol 5 pp. 1193

Charak 1949 *The charak samhita* Delux edn (Jamnagar: Sri Gulab Kunverba Ayurvedic Society) *Sutra*-3/39; *Viman*-8/144, 151; *Chikitsa*-1/2(12), 1/3(3), 1/4(15); 3/258; 6/28; 7/124, 129; 14/124; 21/88; 26/57; 30/75, 82, 108; *Kalp*- 1/8; *Siddh*- 11/24.

Chopra R N, Chopra I C, Handa K L and Kapoor L D 1958 *Indigenous drugs of India* 2nd edn (Calcutta: U.N. Dhur and Sons) pp. 495

Cooke T 1901 *Flora of Presidency of Bombay* (Calcutta: BSI) Vol 1 pp. 512

Dymock W, Warden C J H and Hooper D 1981 *Pharmacographia Indica* (London: Thacker-Spink and Co.) Vol 2 pp. 12

Eldo P M, Patel J D and Shah J J 1977 Occurrence of septate macro-sclereids in the bark of stem of *Anogeissus latifolia* Wall., *Curr. Sci.* **46** 202–203

Gamble J S 1915 *Flora of the Presidency of Madras* (Calcutta: BSI) Part-1 pp. 466

Ganguli S C, Hossain T and Mukherjee B 1945 "Ghatti gum" as an emulsifying agent; *Indian J. Pharmacol.* 7 106–108

Gupta B L 1956 *Forest flora of Chakrata, Dehra Dun and Saharanpur Forest Division, Uttar Pradesh* 3rd edn (Delhi) pp. 238

Johansen D A 1940 *Plant microtechnique* (New York: McGraw Hill) pp. 182–203

Joshi S R 1959 Foliar calcium in *Anogeissus latifolia* Wall.; *J. Indian Bot. Soc.* **3** 93–102

Kay A L 1938 *Microscopical studies of drugs* (London: Bailliere Tindall and Cox) pp. 18

Kirtikar K R and Basu B D 1933 *Indian medicinal plants* (Allahabad: L. N. Basu and Co.) Vol 2 pp. 1034

Kokoski J, Kokoski R and Slama F J 1958 Fluorescence of powdered vegetable drugs under ultra-violet radiation; *J. Am. Pharmacol. Assoc.* **47** 715–717

Mohammed S 1970 *Hamdard pharmacopoeia of eastern medicine* (Karanchi: The Times) pp. 10–15

Nadkarani A K 1954 *Dr K M Nadkarni's Indian materia medica* (Bombay: Popular Book Depot) Vol. 1 pp. 117

Oomachan M 1977 *Flora of Bhopal* (Bhopal: J K Jain Bros.) pp. 162

Randerath K 1963 *Thin layer chromatography* (Boston: Verlag Chemie Academy Press)

Rao J B and Sastry K N S 1960 Isolation of a glycoside from *Anogeissus latifolia* (Dhava-leaves); *Bull. Cent. Leather Res. Inst.* **6** 529

Reddi K K, Rajadurai S, Sastry K N S and Nayudamma Y 1962 Isolation of a naturally occurring trimethyl ether of ellagic acid from Dhava-Bark; *Bull. Cent. Leather. Res. Inst.* **8** 283

Reddi K K, Rajadurai S, Sastry K N S and Nayudamma Y 1962a Isolation of gallic, chebulagic and trigallic acid; *Bull. Cent. Leather. Res. Inst.* **9** 34

Reddi K K, Rajadurai S, Sastry K N S and Nayudamma Y 1964 Studies on Dhava tannins: part V. Biosynthesis of polyphenols of Dhava; *Leather Sci.* **11** 178

Reddi K K, Rajadurai S and Nayudamma Y 1965 Studies on dhava (*Anogeissus latifolia*) tannins II Isolation of gallic, chebulagic and trigallic acids from dhava leaves; *Indian J. Chem.* **3** 129–131

Reddi K K, Rajadurai S and Nayudamma Y 1965a Studies on dhava (*Anogeissus latifolia*) tannins III. polyphenols of bark, sap wood and heart wood of dhava; *Indian J. Chem.* **3** 308–310

Rosenthalar L 1930 *The chemical investigation of plants* (London: G. Bell and Sons Ltd.)

Sushrut 1954 *Sushrut Samhita* (Varanasi: Chowkhamba Sanskrit series office) *Sutra-* 14/36; 16/41; 36/27, 28; 38/11, 19; *Chikista-* 1/85; 2/64; 9/7; 19/33; 21/10; 34/17; *Kalp* 3/9; 6/3, 22; 8/51; *Uttar-* 17/96; 21/47; 24/29; 31/5

Tiagi Y D 1969 Vascular anatomy of the flower of certain species of the Combretaceae; *Bot. Gaz.* **130** 150–157

Vagbhatt 1950 *Astang Hridayam* (Varanasi: Chowkhamba Sanskrit Series office) *Sutra-* 15/7, 19, 32; 24/18; *Sharira* 1/13; *Chikit-* 11/12; 12/7, 24, 29, 41; 18/15; 19/86; *Uttar-* 14/31; 20/12, 39/105

Watt G 1889 *Dictionary of economic products of India.* 2nd edn Vol 1 pp. 256

Abbreviations: cam, cambium; clc, closing cell; cmc, complementary cell; col, collenchyma; cor, cortex; cr, crystal; cr_1, small cluster crystal; cr_2, large sphaerulite; crc, ceratenchyma; cu, cuticle; end, endodermis; ep, epidermis; epb, epiblema; hr, root hair; iph, intraxylary phloem; lbf, libriform xylem fibre; pc, pericycle; pcf, pericyclic fibre; ph, phloem; phd, phelloderm; phf, phloem fibre; phg, phellogen; phl, phellem; php, phloem parenchyma; phr, phloem ray; phr_1, narrow phloem ray: phr_2, broad phloem ray; pi, pith; sie, sieve element; spt, sieve plate; st, sieve tube; stc, stone cell; stg, starch grain; sts, starch sheath; tr, tracheid; tf, tracheid fibre; xy, xylem; xyf, xylem fibre; xyp, xylem parenchyma; xyr, xylem ray; xyr_1, uni-seriate xylem ray; xyr_2, bi-seriate xylem ray; xyv, xylem vessel.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 607-617
© Printed in India.

Cytology of woody species

V K SINGHAL, B S GILL and S S BIR
Department of Botany, Punjabi University, Patiala 147002, India

MS received 13 February 1984

Abstract. Cytological studies have been made on 59 woody species of Polypetalae. Two genera (*Gynocardia odorata*, $n = 23$; *Pahudia martabanica*, $n = 12$) and 9 species (*Talauma candollei*, $n = 19$; *Cratoxylon polyanthum*, $n = 11$; *Sterculia villosa*, $n = 20$; *Grewia hirsuta*, $n = 9$; *Millettia brandisiana*, $2n = 22+0-2B$; *Phanera glauca*, $n = 14$; *Terminalia oliveri*, $n = 12$; *Psidium coriaceum*, $2n = 77$; *P. pumilum*, $n = 11$) are counted for the first time. Additional and/or varied chromosome numbers are recorded for 8 species. Presence of *B*-Chromosomes has been detected in *Crataeva nurvala* ($n = 13+0-3B$), *Erythrina caffra* ($n = 21+0-3B$) and *Millettia brandisiana* ($2n = 22+0-2B$). Existence of some multivalents in the tetraploid taxa of *Eugenia jambolana* ($2n = 44$) and *Hydnocarpus laurifolia* ($2n = 48$) indicates their segmental alloploid nature. But the presence of cent per cent trivalents (11_{III}) in the triploid cytotype of *Eugenia uniflora* ($2n = 33$) reveals the autotriploid nature. Presence of some univalents in diploid taxa of *Millettia brandisiana* ($2n = 22$) may be due to asynapsis and/or desynapsis. Variation in chromosome number in PMCs and some pollen sterility in *Hydnocarpus kurzii* seems to be the consequence of cytomixis. Abnormalities in microsporogenesis in one of the cultivated trees of *Firmiana pallens* ($2n = 40$) are due to spindle irregularities.

Keywords. Chromosome number; alloploid; desynapsis; asynapsis; cytomixis; cytokinesis.

1. Introduction

Attempts on the chromosome counts of forest species growing in the New Forest, Dehra Dun have been made in the past by Rao (1954, 1967) and Nanda (1962). Inspite of the fact that they have determined the chromosome numbers of more than 160 species, several species remain uncovered by them. Also, because of the fact that chromosome countings were made through microtome sectioning, the reports in some cases are contradictory and needs reinvestigation. In view of the above mentioned lacunae and utility of cytological data for future tree breeding programmes, the present studies on the exotic and introduced woody species in the Forest Research Institute, Dehra Dun were undertaken. The present study which includes 59 woody species of Polypetalae is a part of our project on the chromosome analysis of country flora.

2. Material and methods

Materials for meiotic studies were collected from the plants cultivated at Forest Research Institute, Dehra Dun. For meiotic studies, appropriate sized flower buds were fixed in Carnoy's fluid with acetic acid component saturated with iron acetate. Anthers were squashed in 1% acetocarmine and slides were made permanent in euparal. Pollen fertility was estimated with 1:1 glycero-acetocarmine mixture.

3. Observations

Information on chromosome number, ploidy level, pollen fertility and previous reports of the presently investigated species are summarised in table 1. Only species with results of cytological interest are dealt herewith.

3.1 *Crataeva nurvala* Buch. Ham.

Meiotic studies in the species show normal 13 bivalents and 30–35 % pollen sterility. In addition, 8·4 % of the observed PMCs in this cultivated taxon reveal variable (1–3) number of *B*-chromosomes (figure 3), average frequency of which works out to be 0·13. Pairing between two *B*'s is occasional.

3.2 *Hydnocarpus kurzii* (King) Warburg

During meiosis, most of the PMCs show 12 large sized bivalents. The phenomenon of cytomixis which occurs from early prophase I to *T*-II exist in 21·7 % of the PMCs. Consequent to the chromatin transfer, PMCs with increased (figure 6) as well as decreased chromosome numbers result. Extra chromatin material generally lag at *A*-I/*T*-I. Some pollen sterility (13 %) is probably the consequence of cytomixis.

3.3 *H. laurifolia* (Dennst.) Sleumer

The chromosome number $2n = 48$ (figure 7) is confirmed from the PMCs. Multivalents and univalents are present in 8·6 % of the PMCs at diakinesis and *M*-I, with the most common configuration being $2_{IV} + 20_{II}$. Inspite of the formation of quadrivalents (1–4) and trivalents (0–1), involving 12·3 % and 0·2 % of the chromosomes, respectively, distribution of chromosomes during anaphases and pollen fertility is not seriously affected.

3.4 *Firmiana pallens* (Wall. ex King) Koster

Most of the cultivated individuals of this species show the same chromosome number $n = 20$ (figure 10) and regular meiosis. But one tree which is morphologically indistinguishable from the other cultivated trees growing in close vicinity have meiotic irregularities associated with very high pollen sterility (81 %). In this tree, inspite of normal chromosomal pairing, laggards at anaphases and telophases, tripolar distribution of chromosomes at *A*-I (figure 12) and scattered chromosomes during *A*-II (figure 11) are of common occurrence. Laggards are more frequent at *A*-II/*T*-II (38·1 % PMCs) as compared to *A*-I/*T*-I (29·0 % PMCs). Microsporogenesis is also irregular due to the formation of monads (11·8 %, figure 13d, e), dyads (5·5 %, figure 13b, c), triads (7 %, figure 13a) and polyads (14 %, figure 13f). Some of the polyads have large number of microspores few of which are binucleate. Binucleate microspores in dyads and monads are not uncommon.

3.5 *Erythrina caffra* Thunb.

During meiosis 21 bivalents are regularly constituted at *M*-I. Inspite of the regular bivalent formation, laggards are present at *A*-I (figure 21) in some PMCs. Some of the

Table 1. Data on chromosome numbers in the presently studied species.

Taxa	Chromosome number	Ploidy level	Pollen fertility (% age)	Previous reports*
1	2	3	4	5
Dilleniaceae				
Dillenia indica Linn.	$2n = 28$ (figure 1)	$4x$	100	$2n = 48$: Mehra (1976) $2n = 54$: vide Fedorov (1969)
Magnoliaceae				
Magnolia grandiflora Linn.	$n = 57$	$6x$	77	$2n = 112–114$: Morinaga *et al* (1929) $2n = 114$: Janaki Ammal, (1952a); Yamakava vide Fedorov (1969); Koul and Gohil (1973)
Michelia fuscata Blume	$n = 19$	$2x$	100	$2n = 38$: Janaki Ammal (1952a); Nanda (1962); Raven vide Fedorov (1969); Mehra (1976)
Talauma candollei Blume	$n = 19$ (figure 2)	$2x$	–	
Annonaceae				
Polyalthia suberosa Benth. & Hook. f.	$n = 9$	$2x$	100	$2n = 18$: Pancho (1971)
Capparidaceae				
Crataeva nurvala Buch.-Ham. (= *C. lophosperma* Kurz)	$n = 13+0$-$3B$ (figure 3)	$2x$	66	$2n = 26$: Raghavan and Arora (1958)
Flacourtiaceae				
Gynocardia odorata R. Br.	$n = 23$ (figure 4)	$2x$	63	
Homalium tomentosum Benth.	$n = 11$ (figure 5)	$2x$	100	$2n = 20$: Rao (1967)
Hydnocarpus kurzii (King) *Warburg* (= *Taraktogenos kurzii* King)	$n = 12$ (figure 6)	$2x$	87	$2n = 24$: Rao (1954); Mangenot and Mangenot (1962); Nanda (1962)
H. laurifolia (Dennst.) Sleumer (= *H. wightiana* Blume)	$2n = 48$ (figure 7)	$4x$	100	$2n = 24$: Nanda (1962) $2n = 48$: Håmacher (1947)
Oncoba spinosa Forsk.	$n = 11$ (figure 8)	$2x$	100	$2n = 22$: Krishnan (1980)
Pittosporaceae				
Pittosporum rhombifolium A. Cunn. ex Hook.	$n = 12$	$2x$	100	$2n = 24$: Gros (1965)
Guttiferae				
Cratoxylon polyanthum Korth.	$n = 11$ (figure 9)	$2x$	–	
Hypericum chinense Linn.	$n = 21$	$6x$	94	$2n = 42$: Sareen *et al* (1974a, 1980)
Theaceae				
Schima wallichii Choisy	$n = 18$	$2x$	86	$2n = 30$: Malla *et al* (1977a) $2n = 36$: Mehra (1972)
Dipterocarpaceae				
Shorea talura Roxb.	$n = 7$	$2x$	–	$2n = 14$: Roy and Jha (1961, 1965a)

Table 1. (*Continued*)

Taxa	Chromosome number	Ploidy level	Pollen fertility (% age)	Previous reports*
1	2	3	4	5
Sterculiaceae				
Firmiana pallens (Wall. ex King) Koster. (= *Sterculia pallens* Wall. ex King)	$2n = 40$ (figures 10–13)	2*x*	19	$2n = 40$: Nanda (1962); Mehra and Sareen (1973a)
Pterospermum suberifolium Lam.	$n = 19$	2*x*	95	$2n = 38$: Poty and Hamel (1968)
Sterculia acerifolia A. Cunn. (= *Brachychiton acerifolium* F. Muell.)	$n = 20$	2*x*	100	$2n = 40$: Poty and Hamel (1968)
S. villosa Roxb.	$n = 20$ (figure 14)	2*x*	100	
Tiliaceae				
Grewia hirsuta Vahl	$n = 9$ (figure 15)	2*x*	72	
Malpighiaceae				
Byrsonima crassifolia H. B. & K.	$n = 12$ (figure 16)	2*x*	–	$2n = 20$: Nanda (1962) $2n = 24$: Fouët (1966); Bawa (1973) $2n = 28$: Rao (1954)
Malpighia glabra Linn.	$n = 20$	4*x*	90	$2n = 20, 40$: Pandey and Pal (1980) $2n = 40$: Pal (1964); Sarkar *et al* (1980)
Stigmaphyllon periplocaefolium (Desf.) A. Juss.	$n = 10$	2*x*	–	$2n = 20$: Pal (1964)
Rutaceae				
Geijera parviflora Lindl.	$n = 81$	18*x*	*92*	$2n = 108$: Smith-White (1954) $2n = 144$: Rao (1967)
Simaroubaceae				
Ailanthus grandis Prain	$n = 32$ (figure 17)	2*x*	99	$2n = 62$: Mehra and Khosla (1969)
Ochnaceae				
Ochna squarrosa Linn.	$n = 12$ (figure 18)	4*x* (aneu.)	79	$2n = 28$: Mehra and Khosla (1969)
Burseraceae				
Protium serratum (Wall. ex Colebr.) Engl. (= *Bursera serrata* Roxb. ex Colebr.)	$n = 11$	2*x*	100	$2n = 22$: Ghosh (1961, 1969); Mehra (1976)
Meliaceae				
Chukrasia tabularis A. Juss.	$n = 13$	2*x*	67	$2n = 26$: Rao (1967); Mehra (1972); Mehra *et al* (1972c)
Heynea trijuga Roxb. (= *Trichilia connaroides* W. & A.)	$n = 14$	2*x*	100	$2n = 24$: Nanda (1962) $2n = 28$: Rao (1967); Mehra and Sareen (1969); Mehra *et al* (1972c)
Melia dubia Cav. (= *M. composita* Willd.)	$n = 14$	2*x*	29	$2n = 28$: Mehra and Khosla (1969); Mehra (1972); Mehra *et al* (1972c); Datta and Samanta (1977)
Hippocastanacaae				
Aesculus assamica Griff.	$n = 20$	2*x*	97	$2n = 40$: Arora (1961); Rao (1967); Mehra *et al* (1972b)

Table 1. (*Continued*)

Taxa	Chromosome number	Ploidy level	Pollen fertility (% age)	Previous reports*
1	2	3	4	5
Sapindaceae				
Koelreuteria formosana Hayana	$n = 16$ (figure 19)	2*x*	–	$2n = 22$: Bowden (1945b); $2n = 30$: Nanda (1962)
Nephelium longana Camb. (= *Euphoria longana* Lam.)	$n = 15$	2*x*	77	$2n = 30$: Bhaduri and Bose (1949); Guervin (1961a); Mehra *et al* (1972b)
Leguminosae				
Dalbergia latifolia Roxb.	$n = 10$	2*x*	82	$2n = 20$: Atchison (1951); Mehra and Hans (1971, 1972); Mehra (1972); Sanjappa and Dasgupta (1981)
Erythrina caffra Thunb.	$n = 21 + 0$-3B (figures 20, 21)	2*x*	77	$2n = 42$: Atchison (1947b); Krukoff (1969)
Indigofera pulchella Roxb. (= *I. cassioides* Rottl. ex DC.)	$n = 8$	2*x*	–	$2n = 16$: Patil (1958); Bir and Sidhu (1966, 1967); Mitra and Datta (1967); Rao (1967); Bir and Kumari (1977); Sareen and Trehan (1977)
Lonchocarpus neuroscapha Benth.	$n = 11$	2*x*	100	$2n = 22$: Atchison (1949a)
Millettia brandisiana Kurz	$2n = 22 +$ 0-2*B* (figure 22)	2*x*	97	
M. ovalifolia Kurz	$n = 11$	2*x*	100	$2n = 20$: Atchison (1951); Sanjappa and Dasgupta (1977) $2n = 22$: Pal (1960); Sareen *et al* (1974a, 1980); Sanjappa and Dasgupta (1977); Bir and Kumari (1979)
Wisteria sinensis (Sims) DC. (= *W. chinensis* DC.)	$n = 8$	2*x*	51	$2n = 16$: Roscoe (1927); Bir and Kumari (1975)
Bauhinia acuminata Linn.	$n = 13$ (figure 23)	2*x*	100	$2n = 28$: Pantulu (1942); Atchison (1951); Sharma and Raju 1968; Bir and Kumari (1979)
B. galpini N. E Brown	$n = 14$	2*x*	100	$2n = 28$: Atchison (1951); Rao (1954); Sharma and Raju (1968)
Pahudia martabanica Prain	$n = 12$ (figure 24)	2*x*	100	
Peltophorum africanum Sond.	$n = 13$	2*x*	98	$n = 26$: Turner and Fearing (1959); Bir and Kumari (1979)
Phanera glauca Wall. (= *Bauhinia glauca* Wall. ex Benth.)	$n = 14$ (figure 25)	2*x*	57	

Table 1. (*Continued*)

Taxa	Chromosome number	Ploidy level	Pollen fertility (% age)	Previous reports*
1	2	3	4	5
Saraca indica Linn.	$n = 12$	2*x*	86	2*n* = 24: Pantulu (1943); Atchison (1951); Simmonds (1954); Mehra and Hans (1971, 1972); Bir and Kumari (1979); Sanjappa and Dasgupta (1981)
Rosaceae				
Stranvaesia glaucescens Lindl.	$n = 17$	2*x*	–	2*n* = 34: Mehra and Sareen (1969); Mehra *et al* (1973)
Philadelphaceae				
Deutzia scabra Thunb.	$n = 65$	10*x*	–	2*n* = 130: Sax (1931b); Schoennagel (1931)
Combretaceae				
Terminalia arjuna Bedd.	$n = 12$	2*x*	100	2*n* = 24: Janaki Ammal and Sobti (1962); Sanjappa (1979); Gill *et al* (1982)
				2*n* = 26: Sen (1955)
T. myriocarpa Heurck & Muell. Arg.	$n = 12$	2*x*	100	2*n* = 24: Mehra and Khosla (1969, 1972); Gill *et al* (1982)
T. oliveri Brandis	$n = 12$	2*x*	100	
Myrtaceae				
Eugenia jambolana Lam. (= *Syzygium cumini* (Linn.) Skeels	2*n* = 44 (figures 26, 27)	4*x*	86	2*n* = 22: Mehra and Khosla (1969, 1972); 2*n* = 22, 66: Gill (1973); 2*n* = 33, 55: Roy and Jha (1962) 2*n* = 44, 46, 66: Bhaduri and Islam (1949); 2*n* = 66: Mehra and Khosla (1969, 1972); Mehra (1972)
E. uniflora Linn.	$n = 11$ (figure 28)	2*x*	100	2*n* = 22: Bhaduri and Islam (1949); Simmonds (1954)
	2*n* = 33 (figures 29–32)	3*x*	54	2*n* = 33: Singhal *et al* (1983)
Psidium coriaceum Berg.	2*n* = 77 (figure 33)	7*x*	20	
P. pumilum Vahl	$n = 11$ (figure 34)	2*x*	100	
Barringtoniaceae				
Barringtonia acutangula (Linn.) Gaertn.	$n = 13$	2*x*	–	2*n* = 24: Mehra and Singh (1962) 2*n* = 26: Sobti and Singh (1961); Roy and Jha (1965 b; Rao, 1967); Mehra (1976)
Lythraceae				
Heimia myrtifolia Cham. et Schl.	$n = 8$	2*x*	100	2*n* = 16: Dollon and Hamel (1967); Graham (1971)

Table 1. (*Continued*)

Taxa	Chromosome number	Ploidy level	Pollen fertility (% age)	Previous reports*
1	2	3	4	5
Sonneratiaceae				
Duabanga sonneratioides Buch.-Ham. (= *D. grandiflora* (R. Br.) Buch.-Ham.)	$n = 24$	$4x$	100	$2n = 36$: Nanda (1962); $2n = 48$: Roy and Thakur (1961); Rao (1967); Mehra and Bawa (1969); Mehra (1972)

* Darlington and Wylie (1955); Index to plant chromosome numbers (1956–1974); Löve and Löve (1961, 1974, 1975); Fedorov (1969) and selected references from Biological Abstracts.

PMCs (17·7%) in this tree show up to 3*B*-chromosomes (figure 20) with an average frequency of 0·30. Pollen fertility reduces to 77%.

3.6 *Millettia brandisiana* Kurz

Eleven bivalents are regularly consitituted at *M*-I. In a few PMCs, however, two chromosomes are represented as univalents which sometime lag during *A*-I/*T*-I. PMCs with more than one nucleolus of unequal size are also present. Up to 2*B*'s are also recorded in 16% of the PMCs (figure 22) with an average frequency of 0·19.

3.7 *Eugenia jambolana* Lam.

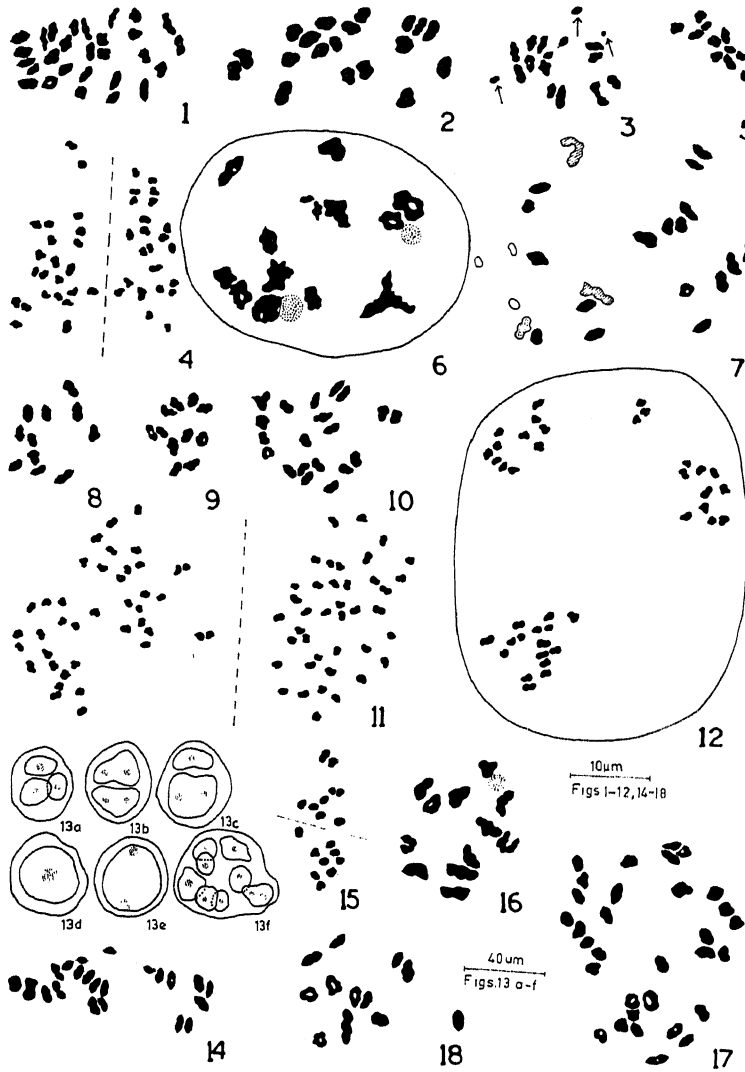
The presently studied cultivated taxon is found to be tetraploid ($2n = 44$) and shows irregular meiosis due to multivalents and univalents (table 2). The most common configuration is with $4_{IV} + 14_{II}$ (figure 26). PMCs with $9_{IV} + 1_{III} + 2_{II} + 1_{I}$ (figure 27) were also observed. Pollen fertility reduces to 86%.

3.8 *Eugenia uniflora* Linn.

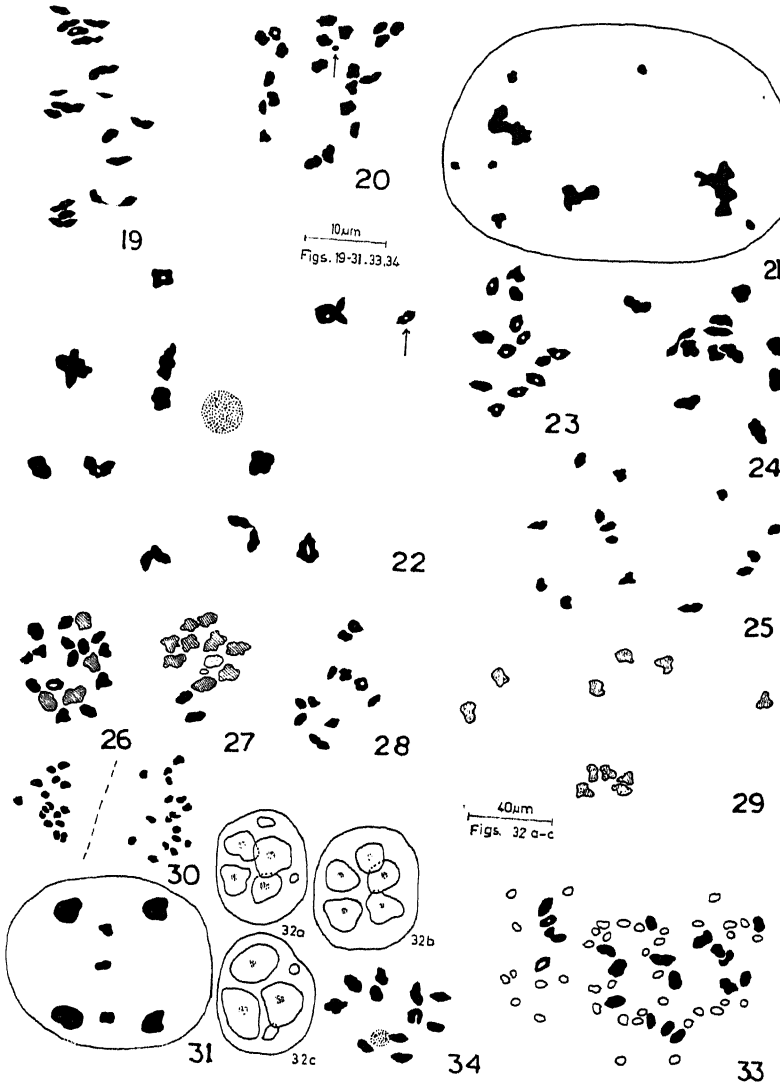
Two cytotypes, diploid ($2n = 22$, figure 28) and triploid ($2n = 33$) are recorded presently from the cultivated individuals. Meiosis in the diploid taxon is normal with

Table 2. Chromosomal associations in *Eugenia jambolana*.

PMCs analysed (12)	*Configurations*			
	IV	III	II	I
Range	0–9	0–1	2–22	0–1
Total	36	1	190	1
Average frequency/PMC	3·0	0·1	16·8	0·1
Percentage of chromosomes involved	27·3	0·7	71·8	0·2



Figures 1–18. Meiosis in pollen mother cells. **1.** *Dillenia indica*, *M*-I with 28_{II}. **2.** *Talauma candollei*, *M*-I with 19_{II}. **3.** *Crataeva nurvala*, *M*-I with 13_{II} and 3*B*'s marked with arrows. **4.** *Gynocardia odorata*, *A*-I showing 23 : 23 distribution. **5.** *Homalium tomentosum*, *M*-I with 11_{II}. **6.** *Hydnocarpus kurzii*, PMC at diakinesis with two nucleoli and extra chromatin material due to cytomixis. **7.** *H. laurifolia*, *M*-I with $2n = 48 = 2_{IV} + 1_{III} + 17_{II} + 3_{I}$. **8.** *Oncoba spinosa*, *M*-I with 11_{II}. **9.** *Cratoxylon polyanthum*, *M*-I with 11_{II}. **10-13.** *Firmiana pallens*. **10.** *M*-I with 20_{II}. **11.** Mixed *A*-II with 40 : 40 chromosomes in two groups. **12.** *A*-I showing tripolar distribution of chromosomes. **13a-f.** Abnormal microsporogenesis. **a.** triad. **b.** dyad with two nuclei in each unit. **c.** dyad with unequal units and large unit showing two nuclei. **d.** monad. **e.** monad with two nuclei. **f.** polyad with one unit showing two nuclei. **14.** *Sterculia villosa*, *M*-I with 20_{II}. **15.** *Grewia hirsuta*, *A*-I showing 9 : 9 distribution. **16.** *Banisteria laevifolia*, diakinesis showing 12 bivalents. **17.** *Ailanthus grandis*, *M*-I with 32_{II}. **18.** *Ochna squarrosa*, *M*-I with 12_{II}.



Figures 19–34. Meiosis in pollen mother cells. **19.** *Koelreuteria formosana*, *M*-I with 16_{II}. **20,21.** *Erythrina caffra*. **20.** PMC at *M*-I showing $21_{II}+1B$. **21.** Laggards at late *A-I*. **22.** *Millettia brandisiana*, diakinesis showing $11_{II}+1B$. **23.** *Bauhinia acuminata*, *M*-I with 13_{II}. **24.** *Pahudia martabanica*, *M*-I with 12_{II}. **25.** *Phanera glauca*, *M*-I with 14_{II}. **26,27.** *Eugenia jambolana*. **26.** *M*-I with $2n = 44 = 4_{IV}+14_{II}$. **27.** *M*-I with $2n = 44 = 9_{IV}+1_{III}+2_{II}+1_{I}$. **28–32.** *E. uniflora*. **28.** Diploid cytotype. PMC at *M*-I showing 11_{II}. **29–32.** Triploid cytotype. **29.** *M*-I with $2n = 33 = 11_{III}$. **30.** *A*-I showing 16:17 chromosome distribution. **31.** *T*-II showing laggards. **32 a–c.** Abnormal microsporogenesis. **a.** tetrad with two micropollens. **b.** polyad. **c.** triad with two micropollens. **33.** *Psidium coriaceum*, *M*-I with $2n = 77 = 19_{II}+39_{I}$. **34.** *P. pumilum*, *M*-I with 11_{II}.

cent per cent pollen fertility. But in the triploid it is highly irregular and is characterised by the presence of trivalents and univalents, unequal distribution of chromosomes, laggards, etc. Analysis of chromosome association reveals that the number of trivalents, bivalents and univalents ranges from 0–11, 0–3 and 0·3 with the average

frequency being 10·6, 0·4 and 0·4 respectively. The most common configuration is of 11 trivalents (62·7 % PMCs, figure 29) which is the maximum possible number of trivalents in a triploid with $2n = 33$. Distribution of chromosomes during *A*-I is quite irregular with the most common type being 22:11. PMCs with 16:17 (figure 30), 15:18, 14:19 and 13:20 distribution are also not uncommon. Laggards which vary from 1–5 are recorded in about 50 % of the PMCs at *A*-I/*T*-I and 60 % of the PMCs at *A*-II/*T*-II (figure 31). In some of the PMCs, chromosomes remain scattered and fail to reach the poles. Consequent to this, microsporogenesis is quite abnormal resulting in the formation of tetrads (41·3 %, figure 32a) and triads (5·8 %, figure 32c) with micropollen and polyads (9·0 %, figure 32b). Pollen malformation which is quite high (46 %) includes 25 % micropollen.

3.9 *Psidium coriaceum* Berg

The chromosome count of $2n = 77$ (figure 33) for the species is confirmed from the microsporocytes. Meiosis in the taxa is highly abnormal leading to high pollen sterility (80 %). Analysis of chromosomal association reveals that the number of bivalents and univalents ranges from 17–23 and 31–43, respectively, with average frequency of $20{\cdot}6_{II} + 35{\cdot}7_{I}$.

4. Discussion

Consequent to the present investigations on the woody species from the New Forest, Dehra Dun already covered by some workers (Rao 1954, 1967; Nanda 1962), two genera (*Gynocardia odorata*, $n = 23$; *Pahudia martabanica*, $n = 12$) and 9 species (*Talauma candollei*, $n = 19$; *Cratoxylon polyanthum*, $n = 11$; *Sterculia villosa*, $n = 20$; *Grewia hirsuta*, $n = 9$; *Millettia brandisiana*, $2n = 22 + 0{-}2B$; *Phanera glauca*, $n = 14$, *Terminalia oliveri*, $n = 12$; *Psidium coriaceum*, $2n = 77$ and *P. pumilum*, $n = 11$) are counted for the first time. Additional and/or varied chromosome numbers are recorded in 12 species which include 8 species already counted from the same area by earlier workers. The information along with comments about these reports are given in table 3.

Presence of *B*-chromosomes has been noticed in *Crataeva nurvala* ($2n = 26 + 0{-}3B$), *Erythrina caffra* ($2n = 42 + 0{-}3B$) and *Millettia brandisiana* ($2n = 22 + 0{-}2B$). Incidentally, all the three species are at diploid level.

As many as 6 species, namely, *Hydnocarpus laurifolia* (2*x*, 4*x*), *Malpighia glabra* (2*x*, 4*x*), *Geijera parviflora* (12*x*, 16*x*, 18*x*), *Eugenia jambolana* (2*x*, 3*x*, 4*x*, 5*x*, 6*x*), *E. uniflora* (2*x*, 3*x*) and *Duabanga sonneratioides* (3*x*, 4*x*) show intraspecific polyploid cytotypes. Among these, additional cytotypes have been recorded for *H. laurifolia* ($2n = 4x = 48$), *Geijera parviflora* ($2n = 18x = 162$) and *Eugenia uniflora* ($2n = 3x = 33$). Constitution of multivalents in tetraploid taxa of *Hydnocarpus laurifolia* ($2n = 48$) and *Eugenia jambolana* ($2n = 44$) indicates the segmental alloploid nature. On the other hand, meiosis in the triploid cytotype of *Eugenia uniflora* ($2n = 33$) is characterized by cent per cent trivalent (11_{III}) formation on the basis of which Singhal *et al* (1983) suggested the cytotype to be an autotriploid originated through the involvement of unreduced (diploid) and reduced (haploid) gametes. Meiosis in *Psidium coriaceum* ($2n = 77$) which is a very high polyploid (7*x*) is also abnormal. The presence of high frequency of univalents and complete absence of

Table 3. Variable chromosome records.

Taxa	Present record ($2n =$)	Previous reports ($2n =$)	Comments
*Homalium tomentosum**	22	20	Aneuploidy at diploid level.
*Hydnocarpus laurifolius**	48	24,48	First report of tetraploid cytotype from India.
*Byrsonima crassifolia**	24	20,24,28	Refutes the earlier reports of $2n = 20,28$.
*Geijera parviflora**	162	108,144	Additional report of polyploid cytotype (18x).
Ailanthus grandis	64	62	Aneuploidy at diploid level.
Ochna squarrosa	24	28	Aneuploidy at tetraploid level.
Dillenia indica	56	48,54	First report of tetraploid cytotype on $x = 14$.
*Heynea trijuga**	28	24	Earlier report of $2n = 24$ proves to be erroneous.
*Koelreuteria formosana**	32	22,30	First report of cytotype with $n = 16$.
Bauhinia acuminata	26	28	Aneuploid at diploid level.
*Duabanga sonneratioides**	48	36,48	Earlier report of $2n = 48$ confirmed.
*Eugenia uniflora**	22,33	22	First report of triploid cytotype and intraspecific polyploidy for the species.

* Species earlier counted from the New Forest, Dehra Dun.

multivalents in this unbalanced polyploid indicate its alloploid nature. Existence of only two univalents in some PMCs during diakinesis and *M*-I in *Millettia brandisiana* ($2n = 22$) which is a normal diploid might be the consequence of asynapsis and/or desynapsis.

The phenomenon of cytomixis with the involvement of chromatin transfer and constitution of hypo-and hyperploid PMCs is confirmed in *Hydnocarpus kurzii*. Since the species is normal diploid, the phenomenon seems to be under some genetic control as proposed by Brown and Berkre (1974) and Omara (1976).

Meiotic abnormalities like scattered or unoriented chromosomes, unequal distribution and laggards at anaphases, partial or complete failure of cytokinesis in one of the cultivated trees of *Firmiana pallens* which is a normal diploid ($2n = 40$) appear to be the result of some sort of spindle irregularities. Since both the trees with and without such irregularities grow side by side under the same conditions, these might be under some genetic factors as is the case with *Cephalotaxus drupacea* var. *pedunculata* (Khoshoo 1957) and *Grewia hainesiana* (Singhal *et al* 1982).

Acknowledgement

Thanks are due to UGC, New Delhi, for financial assistance.

References

Brown W V and Berkre E M 1974 *Textbook of cytology* (Missouri: Ind. ed. Mosby. Saint. Louis)

Darlington C D and Wylie A P 1955 *Chromosome atlas of cultivated plants* (London: George Allen and Unwin Ltd.)

Fedorov An A (ed.) 1969 *Chromosome numbers of flowering plants.* (Leningrad: Acad. Sci. USSR Komarov Botanical Inst.)

Khoshoo T N 1957 Cytology of conifers. III. Partial failure of meiotic spindle in *Cephalotaxus drupacea* var. *pedunculata; Cytologia* **22** 80–89

Löve A and Löve D 1961 Chromosome numbers of central and north-west European plant species; *Opera Bot.* (Stockholm: Almqvist and Wiksell)

Löve A and Löve D 1974 *Cytotaxonomical atlas of the Slovenian Flora* Lehre, J. Cramer, 3301

Löve A and Löve D 1975 *Cytotaxonomical atlas of Arctic flora* Vatuz, J. Cramer Fl. 9490

Nanda P C 1962 Chromosome numbers of some trees and shrubs; *J. Indian Bot. Soc.* **41** 271–277

Omara M K 1976 Cytomixis in *Lolium perenne*; *Chromosoma* **55** 267–271

Rao H S 1954 Chromosome numbers in forest plants; *Ind. For.* **80** 551–552

Rao H S 1967 Chromosome counts of new forest plants; *Ind. For.* **93** 243–254

Singhal V K, Gill B S and Bir S S 1982 Cytology of some Indian species of *Grewia* Linn. (Tiliaceae); *J. Tree Sci.* **1** 88–97

Singhal V K, Gill B S and Bir S S 1983 Natural triploidy in *Eugenia uniflora* Linn; *Sci. Cult.* **49** 132–133

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 619-626
© Printed in India.

Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India)

S S BIR and M I S SAGGOO
Department of Botany, Punjabi University, Patiala 147 002, India

MS received 5 May 1984; revised 10 January 1985

Abstract. Chromosomal analysis of 33 species of Labiatae from South India have indicated that 23·53 % are polyploids. Several taxa are investigated for the first time. Analysis of results in totality for the region indicates that shrubby and perennial species have higher incidence of polyploidy. Polybasic nature of the family as well as the investigated taxa is quite apparent and this indicates the role of eu- and aneuploidy in evolution of various taxa in the family.

Keywords. Chromosome numbers; Labiatae; polyploidy; polybasic.

1. Introduction

South India is one of the major centres of distribution of members of family Labiatae in India since it supports over 139 species (Mukerjee 1940). Cytologically, Labiates of South India have not hitherto attracted much attention. Barring the chromosome number reports by Vembu and Sampathkumar (1978, 1980), Cherian and Kuriachan (1981), and Krishnappa and Indiramma (1982), no significant work has been done so far. Therefore, the present studies were carried out as a part of our project on Indian Bicarpellatae.

2. Material and methods

Materials for the present studies were collected from Kodaikanal (10° 13′N lat. and 77° 32′ E long.), a hill resort in Madurai district of Tamil Nadu, and its surroundings between altitudinal range of 300–2400 m. The specific localities for the materials of various taxa are mentioned in table 1. Meiotic studies were made following the usual acetocarmine squash techniques after fixations of young flower buds in Carnoy's fluid. Voucher specimens are preserved in PUN.

3. Observations

Presently, 33 species (34 taxa) belonging to 13 genera are chromosomally analysed and the obtained information is provided in table 1. In each case several populations were studied so as to arrive at the exact chromosome number and follow the meiotic process leading to pollen formation. In majority of the cases meiosis is normal leading to the formation of normal, apparently, viable pollen. However, in few cases, certain meiotic abnormalities have been observed as per details that follow. Information on palynology has been published elsewhere (Saggoo and Bir 1983).

Table 1. Chromosome numbers in South Indian Labiatae.

Name of Taxa 1	Localities† 2	Chromosome numbers+ 3	Ploidy level 4	PUN Accession number(s) 5
Anisomeles indica O. Ktze.	I*a*	$n = 17$ (figure 1)	Diploid	27036
A. malabarica R. Br.	I*b*, *c*	$n = 17$ (figure 2)	Diploid	27247, 26931
Calamintha umbrosa Fisch. & Mey	I*d*	$n = 19$ (figure 3)	Tetraploid (aneuploid)	27117
Coleus aromaticus Benth.	I*e*	$n = 17$ (figure 4)	Diploid	26915
C. blumei Benth.	I*f*	$n = 24$	Tetraploid	27844
C. forskohlii Briq.	I*f*, *g*	$n = 15$ (figure 5)	Diploid	27031, 27254
C. malabaricus Benth.	I*h*	$n = 14$	Diploid	26958
Dysophylla auricularia Bl.**	I*a*	$n = 17$ (figure 20)	Hexaploid (aneuploid)	27252
Hyptis suaveolens Poit.	I*a*	$n = 14$ (figure 6)	Diploid	27130
Micromeria biflora Benth.	I*i*	$n = 15$	Diploid	27123
Ocimum adscendens Willd.	I*e*	$n = 11$ (figure 7)	Diploid	26964
O. basilicum Linn.	I*e*	$n = 24$	Tetraploid	27237
O. canum Sims.	I*e*	$n = 13$	Diploid	26961
O. kilimandscharicum Guerke	I*j*	$n = 38$	Tetraploid	27265
O. sanctum Linn. var. *hirsuta* Hook. f.*	I*c*	$n = 18$ (figure 8)	Tetraploid	26966
Orthosiphon glabratus Benth.	I*k*	$n = 13$	Diploid	26941
O. pallidus Royle	I*l*	$n = 14$ (figure 9)	Diploid	26929
O. tomentosus Benth.*	I*b*	$n = 13$ (figure 21)	Diploid	26940
var. *tomentosa* Hook. f.*	I*f*	$n = 13$ (figure 10)	Diploid	27250
Plectranthus fruticosus Wight**	I*i*	$n = 28$ $2n = 56$ (figure 22)	Tetraploid	26868
P. macraei Benth.*	I*g*, *f*	$n = 12$ (figure 11)	Diploid	26969, 27032
P. mollis Spreng.	I*m*	$n = 14$ (figure 12)	Diploid	27259
P. nilghiricus Benth.	I*i*	$n = 12$ (figure 13)	Diploid	26972
P. stocksii Hook. f.*	I*n*	$n = 12$ (figure 24)	Diploid	26974
P. wightii Benth.	I*n*, *o*	$n = 12$ (figure 14)	Diploid	26930, 26925
Pogostemon paludosus Benth.*	I*i*, *p*	$n = 17$ (figure 15)	Diploid	26905, 27042
P. pubescens Benth.*	I*h*	$n = 16$ (figure 16)	Diploid	20827
Prunella vulgaris Linn.	I*q*	$n = 14$	Diploid	27172
Salvia coccinea Juss.	I*j*	$n = 11$ (figure 17)	Diploid	26872

Table 1 (*Continued*)

Name of Taxa 1	Localities† 2	Chromosome numbers+ 3	Ploidy level 4	PUN Accession number(s) 5
S. grahmi Benth.	I*p*, II*a*	$n = 11$ (figure 18)	Diploid	26883, 27241
S. splendens Sello	I*n*	$n = 22$	Tetraploid	27262
Scutellaria colebrookiana Benth.*	I*f*	$n = 13$ (figure 25)	Diploid	26918
S. violacea Heyne*	I*i*	$n = 13$ (figure 19	Diploid	26895
S. wightiana Benth.*	III*a*	$n = 13$ (figure 26)	Diploid	26919

† Localities: I, Kodaikanal; *a*, Perumalmalai, 1600m; *b*, Amsapuram, 300m; *c*, Kodaikanal Road, 250m; *d*, Shembaganur, 1850m; *e*, Fall's view, 900m; *f*, Palmalai, 1200m; *g*, Kodaikanal town, 2050m; *h*, Tiger shola, 1800m; *i*, Bear shola, 1900m; *j*, Malayadumparai, 1500m; *k*, Pholather, 1100m; *l*, Palni, 300m; *m*, Uttoo, 1200m; *n*, Pillar rocks, 2200m; *o*, Vellagavie, 1600m; *p*, Kodai shola, 2100m; *q*, Berijam, 2400m. II. Ootacamund: *a*, Botanical Garden, 2400m. III. Kumali (Kerala): *a*, Thekkadi, 950m.

+ For previous reports reference is made to Darlington and Wylie (1955), Löve and Löve (1961, 1974, 1975), Fedorov (1969), Index to plant chromosome numbers (1956 onwards), IOPB chromosome number reports (1965 onwards) and selected references from Biological Abstracts up to the end of 1983.

* Worked out for the first time.

** Different reports.

3.1 *Plectranthus fruticosus*

In *P. fruticosus* ($2n = 56$) meiosis is highly abnormal with 83·1 % of the PMCs showing 4–6 univalents (figure 22). Average frequency of bivalents and univalents per PMC is 25·81 % and 4·37 %, respectively (table 2). The most common configuration is $25_{II} + 6_{I}$ being represented in 32·11 % of PMCs at *M*-I. Other chromosomal configurations are $26_{II} + 4_{I}$ (figure 22) and 28_{II} in 30·99 % and 16·99 %, respectively, of the observed PMCs at *M*-I. Invariably univalents lag at *A*-I/*T*-I. Few (4·32 %) PMCs show formation of bridges or lagging of one bivalent along with univalents (figure 23). Laggards form micronuclei which lead to polyad formation in 81·6 % of the PMCs resulting in only 37·8 % fertile and apparently viable pollen grains.

3.2 *Scutellaria colebrookiana*

In *S. colebrookiana* ($n = 13$) 26·19 % of the PMCs observed at *M*-I show precocious disjunction of one bivalent but without affecting the future course of meiosis. In few PMCs of another species of the genus, *S. wightiana* ($n = 13$), 1–2 bivalents show late disjunction at *A*-I (figure 27). Here, too, the further course of meiosis is normal.

4. Discussion

Cumulative cytological data on the genus *Ocimum* reveal its polybasic nature ($x = 8$, 11, 12, 13, 19). Singh and Sharma (1982) suggested $x = 8$ and 12 as the two primary

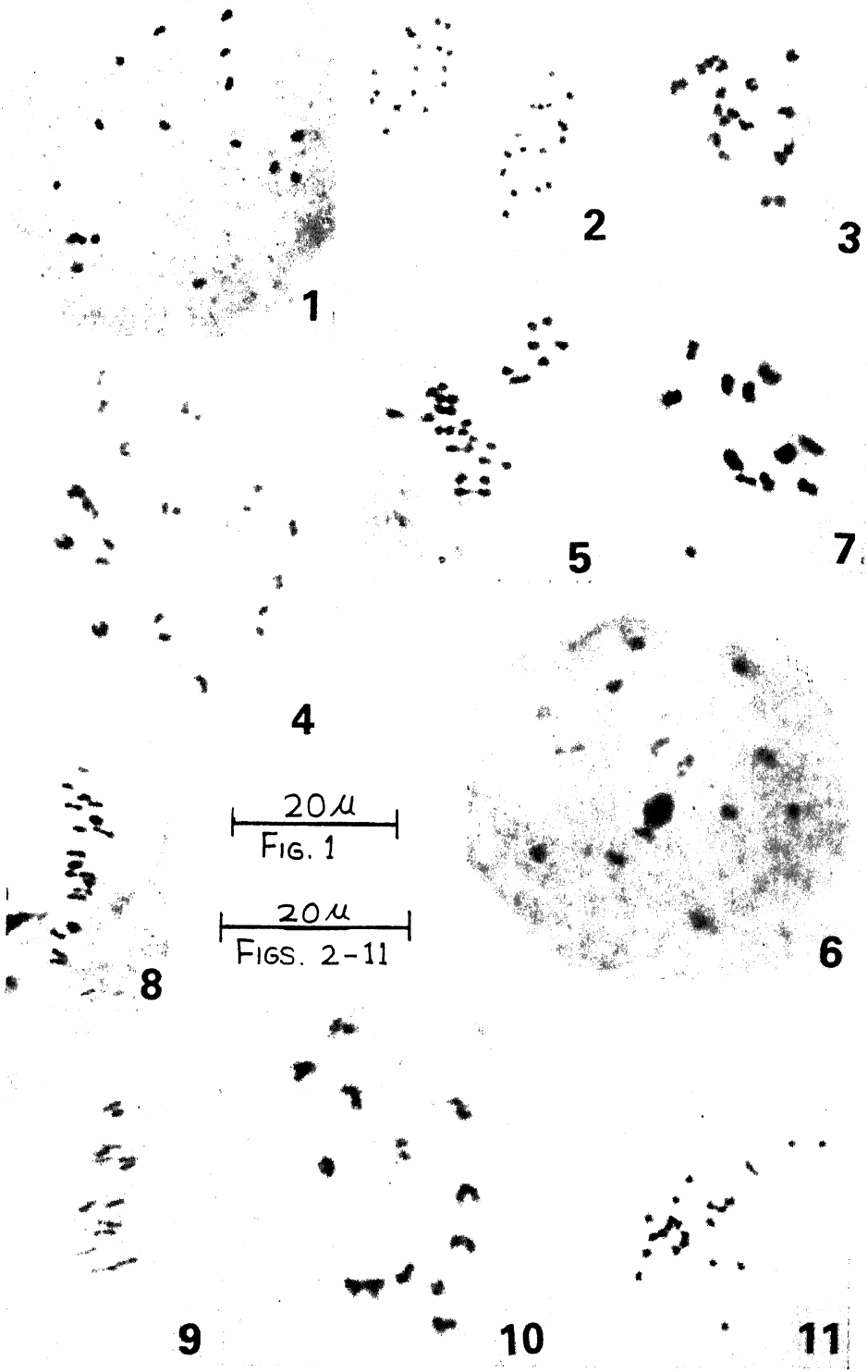

1
2
3
4
5
7
6
8
9
10
11
20 μ
FIG. 1
20 μ
FIGS. 2-11

Table 2. Chromosomal associations at *M*-I in *Plectranthus fruticosus* Wight.

Number of PMCs	Percentage	Univalents	Bivalents
37	52·11	6	25
22	30·99	4	26
12	16·90	0	28
Total: 71	100·00	310	1833
Average/PMC	-	$4{\cdot}37_{I}$	$25{\cdot}81_{II} = 2n = 56$

basic numbers. Among the presently investigated species, *O. adscendens* ($2n = 22$) and *O. canum* ($2n = 26$) are diploids based on $x = 11$ and 13, respectively, while *O. basilicum* ($2n = 48$) and *O. kilimandscharicum* ($2n = 78$) are tetraploids based on $x = 12$ and $x = 19$, respectively. *O. sanctum* var. *hirsuta* with $n = 18$ is an aneuploid at tetraploid level.

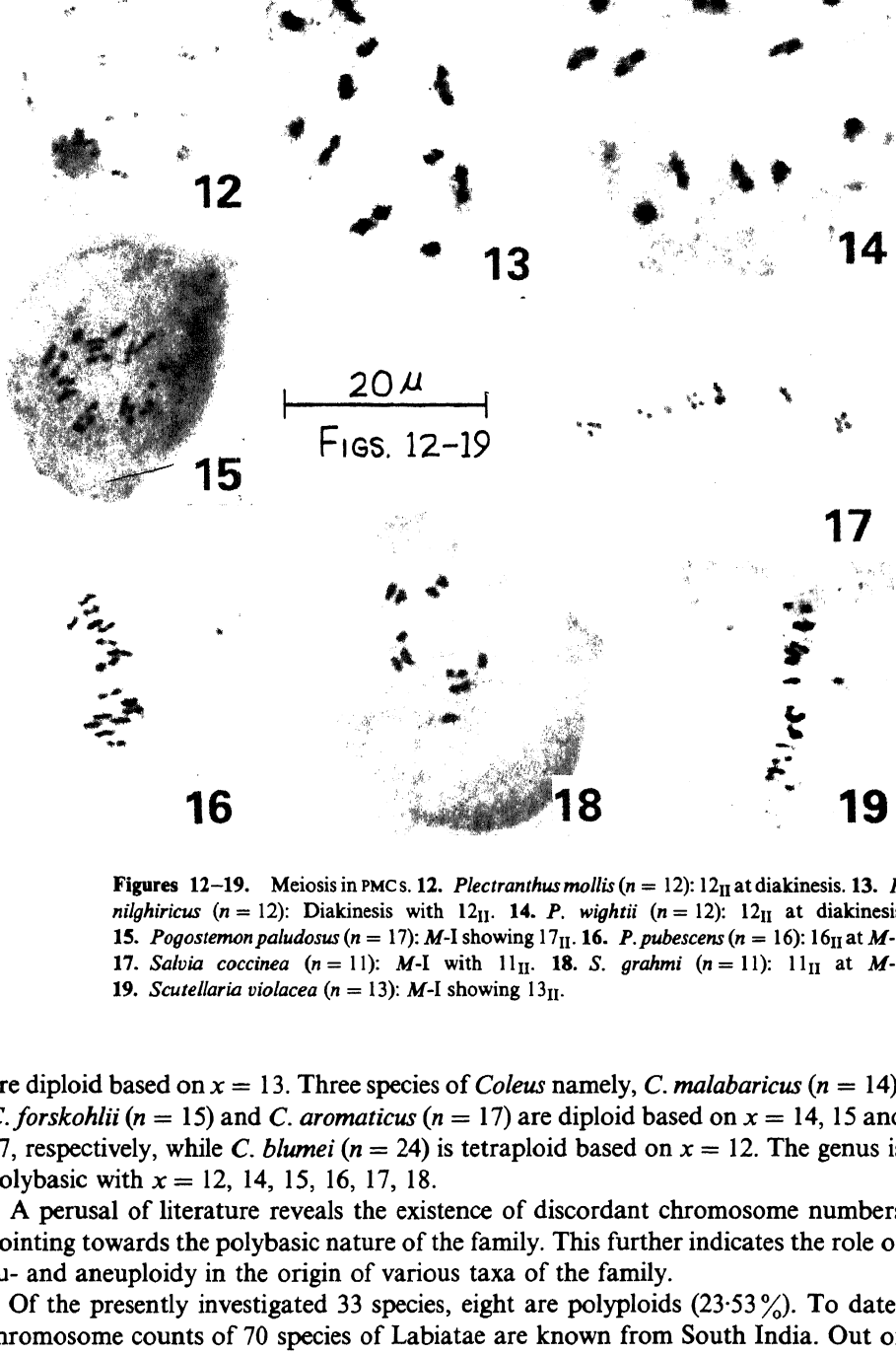
All the three investigated species of the genus *Orthosiphon* are at diploid level. *O. glabratus* and *O. tomentosus* are based on $x = 13$ while *O. pallidus* is based on $x = 14$. So far, eight species of the genus are known chromosomally, clearly indicating the polybasic nature of the genus ($x = 11$, 12, 13, 14).

Both the species of *Pogostemon*, namely, *P. pubescens* ($n = 16$) and *P. paludosus* ($n = 17$) are diploid based on $x = 16$ and 17, respectively. Gill (1971) is of the view that $x = 8$ is the original base number for the genus, however, Bir and Saggoo (1982) suggested $x = 16$ and 17, though of secondary origin. Out of six species of *Plectranthus*, four species *viz. P. macraei* ($n = 12$), *P. nilghiricus* ($n = 12$), *P. stocksii* ($n = 12$) and *P. wightii* ($n = 12$) are diploid based on $x = 12$. The other two *viz. P. mollis* and *P. fruticosus* are based on $x = 14$. The former with $n = 14$ is diploid while the latter with $n = 28$, is tetraploid. This is an unbalanced polyploid since the presence of up to 6_{I_s} is of frequent occurrence. The existence of the tetraploid cytotype of *P. fruticosus* is recorded for the first time. Earlier, de Wet (1958) and Morton (1962) reported from Africa diploid plants of the species with $2n = 28$.

Dysophylla auricularia with $2n = 34$ is also studied for the first time from India and is in line with the earlier report by Chaung *et al* (1963) from Taiwan. Hsu (1967), also from Taiwan, however, recorded $2n = 12$ for the species. Taking $x = 6$ as its base number the presently worked out taxon is an aneuploid at hexaploid level.

The genus *Scutellaria* is polybasic with base number $x = 8$, 11, 12, 13, 15 and 17 (Gill 1970). The presently studied species *viz. S. colebrookiana*, *S. violacea* and *S. wightiana*

Figures 1–11. Meiosis in pollen mother cells (PMCs). **1.** *Anisomeles indica* ($n = 17$): *M*-I showing 17_{II}. **2.** *A. malabarica* ($n = 17$): *A*-I showing 17+17 chromosomes. **3.** *Calamintha umbrosa* ($n = 19$): 19_{II} at diakinesis. **4.** *Coleus aromaticus* ($n = 17$): Diakinesis showing 17_{II}. **5.** *C. forskohlii* ($n = 15$): *M*-I with few bivalents already disjuncted. **6.** *Hyptis suaveolens* ($n = 14$): 14_{II} at diakinesis. **7.** *Ocimum adscendens* ($n = 11$): *M*-I with 11_{II}. **8.** *O. sanctum* var. *hirsuta* ($n = 18$): *M*-I showing 18_{II}. **9.** *Orthosiphon pallidus* ($n = 14$): 14_{II} at *M*-I. **10.** *O. tomentosus* var. *tomentosa* ($n = 13$): Diakinesis showing 13_{II}. **11.** *Plectranthus macraei* ($n = 11$): Mixed *A*-I showing 24 chromosomes.

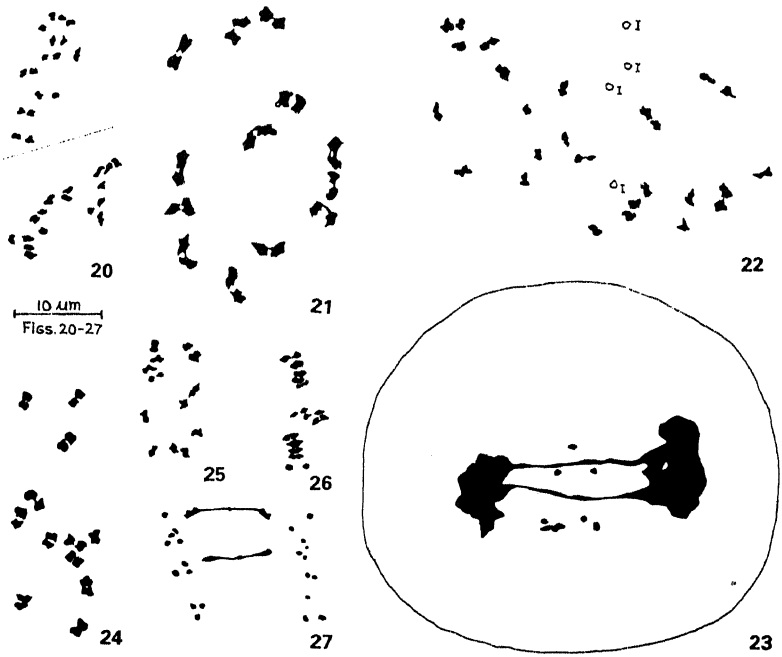


Figures 12–19. Meiosis in PMCs. **12.** *Plectranthus mollis* ($n = 12$): 12_{II} at diakinesis. **13.** *P. nilghiricus* ($n = 12$): Diakinesis with 12_{II}. **14.** *P. wightii* ($n = 12$): 12_{II} at diakinesis **15.** *Pogostemon paludosus* ($n = 17$): *M*-I showing 17_{II}. **16.** *P. pubescens* ($n = 16$): 16_{II} at *M*- **17.** *Salvia coccinea* ($n = 11$): *M*-I with 11_{II}. **18.** *S. grahmi* ($n = 11$): 11_{II} at *M*- **19.** *Scutellaria violacea* ($n = 13$): *M*-I showing 13_{II}.

are diploid based on $x = 13$. Three species of *Coleus* namely, *C. malabaricus* ($n = 14$) *C. forskohlii* ($n = 15$) and *C. aromaticus* ($n = 17$) are diploid based on $x = 14$, 15 and 17, respectively, while *C. blumei* ($n = 24$) is tetraploid based on $x = 12$. The genus is polybasic with $x = 12$, 14, 15, 16, 17, 18.

A perusal of literature reveals the existence of discordant chromosome numbers pointing towards the polybasic nature of the family. This further indicates the role of eu- and aneuploidy in the origin of various taxa of the family.

Of the presently investigated 33 species, eight are polyploids (23·53%). To date chromosome counts of 70 species of Labiatae are known from South India. Out of these only 12 (17·14%) are polyploids. Intraspecific aneuploid cytotypes have been recorded in 14·28% of Labiates from South India. So far, intraspecific polyploidy is reported only in *Coleus blumei* ($2x$, $4x$, $6x$) by Reddy (1952). Correlating the incidence



Figures 20–27. Meiosis in PMCs. **20.** *Dysophylla auricularia* ($n = 17$): *M*-II with 17 + 17 chromosomes. **21.** *Orthosiphon tomentosus* ($n = 13$): 13_{II} at diakinesis. **22–23.** *Plectranthus fruticsus* ($2n = 56$): 22, *M*-I with 26_{II} and 4_{I}; **23.** *T*-I with bridges along with one bivalent and other laggards. **24.** *P. stocksii* ($n = 12$): 12_{II} at *M*-I. **25.** *Scutellaria colebrookiana* ($n = 13$): *M*-I with 13_{II}. **26, 27.** *S. wightiana* ($n = 13$) **26.** *M*-I showing 13_{II}. **27.** *A*-I with late disjunction in two bivalents.

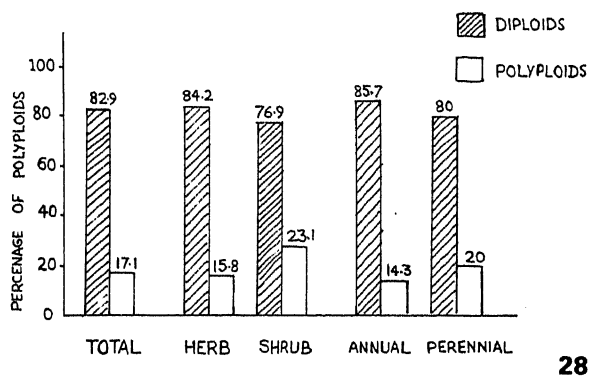


Figure 28. Correlation of the life-form and polyploidy in the South Indian Labiatae.

of polyploidy with life form, it is quite clear that shrubby and perennial species have higher incidence of polyploidy as compared to the herbaceous and annual members (figure 28).

Acknowledgements

One of the authors (MISS) is grateful to CSIR., New Delhi for the award of a Research Fellowship.

References

Bir S S and Saggoo M I S 1982 Cytology of some members of Labiatae from Central India; *Proc. Natl. Acad. Sci.* **B52** 107–112

Cherian M and Kuriachan P I 1981 In: Chromosome number reports LXXII; *Taxon* **30** 694–708

Chaung T I, Chao C Y, Hu W W L and Kwan S C 1963 Chromosome number of vascular plants of Taiwan 1; *Taiwania* **1** 51–66

Darlington C D and Wylie A P 1955 *Chromosome atlas of flowering plants* (London: George Allen and Unwin Ltd.) pp. i-xx, 1–519

Fedorov An A (ed.) 1969 *Chromosome numbers of the flowering plants* Academy of Sciences of the USSR Komarov Botanical Institute Leningrad pp. 1–928

Gill L S 1970 Cytological observations on West Himalayan Labiatae: Tribe-Stachydeae; *Phyton* (*Buneos Aires*) **17** 177–184

Gill L S 1971 Cytology of West Himalayan Labiatae: Tribe-Satureineae; *Caryologia* **24** 203–207

Hsu C C 1967 Preliminary chromosome studies on the vascular plants of Taiwan (1); *Taiwania* **14** 117–130

Krishnappa D G and Indiramma B 1982 In: IOPB Chromosome number reports LXXV; *Taxon* **31** 342–368

Löve A and Löve D 1961 Chromosome numbers of central and north west European plant species; *Opera Bot.* **5** 1–581

Löve A and Löve D 1974 *Cytotaxonomical atlas of the Slovenian flora* J. Cramer. FL-3301 Lehre pp. i-xx, 1–1241

Löve A and Löve D 1975 *Cytotaxonomical atlas of the Arctic flora*; J. Cramer FL-9490 Vaduz pp. i-xxiii, 1–598

Morton J K 1962 Cytotaxonomic studies on the west African Labiatae; *J. Linn. Soc. London Bot.* **58** 231–283

Mukerjee S K 1940 A revision of the Labiatae of Indian Empire; *Rec. Bot. Surv. India* **14** 1–228

Reddy N S 1952 Chromosome numbers in *Coleus; J. Hered.* **43** 233–237

Saggoo M I S and Bir S S 1983 Cytopalynological studies on Indian members of Acanthaceae and Labiatae; *J. Palynol.* **19** 243–277

Singh T P and Sharma A K 1982 Chromosome evolution in *Ocimum*; *Nucleus* **25** 59–64

Vembu B and Sampathkumar R 1978 In: IOPB Chromosome number reports LXII; *Taxon* **27** 519–535

Vembu B and Sampathkumar R 1980 In: Chromosome number reports LXVI; *Taxon* **29** 163–169

de Wet J M J 1958 Chromosome numbers in *Plectranthus* and related genera: *S. Afr. Sci.* **54** 153–156

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 627-632
© Printed in India.

Seasonal growth, reproduction and spore shedding in *Pterocladia heteroplatos*

N KALIAPERUMAL* and M UMAMAHESWARA RAO

Department of Botany, Andhra University, Waltair 530003, India

* Regional Centre of Central Marine Fisheries Research Institute, Marine Fisheries, Tamil Nadu 623520, India

MS received 21 November 1984

Abstract. Results on seasonal growth, fruiting behaviour and spore output in *Pterocladia heteroplatos* are presented. Populations of *P. heteroplatos* were recorded in all months of the year with a maximum development of plants from November to January. Tetrasporophytes occurred throughout the year without any seasonal variation in their abundance but carposporophytes were rarely found in natural population. The tetraspore and carpospore outputs ranged from 1427–7 94 055 and 39 996–1 67 040 spores/g fresh weight of the plant respectively. Seasonal changes were not found in the formation and liberation of reproductive bodies in *P. heteroplatos*.

Keywords. Spore shedding; reproduction, growth; seasonal changes; Pterocladia heteroplatos.

1. Introduction

Species of *Pterocladia* (Gelidiaceae) are used as a source of agar-agar (Boney 1965). *P. heteroplatos* grows at Visakhapatnam (Rao and Kaliaperumal 1980). Results obtained on the yield and properties of agar extracted from *P. heteroplatos*, growing at Visakhapatnam (Rao and Kaliaperumal 1980) have been published recently. The present paper reports the biological data collected on the growth and reproductive phenology and spore shedding for a period of two and a half years from September 1976 to February 1979.

2. Material and methods

P. heteroplatos growing in the infralittoral fringe zone was collected randomly at fortnightly intervals from two stations along the Visakhapatnam coast (17° 41′ 45″ N and 83° 16′ 22″ E). Twenty to twenty-five tufts of *P. heteroplatos* were brought to the laboratory in polythene bags containing sea-water and used for growth analysis and spore liberation experiments. Details of the methods followed for analysing the growth and fruiting behaviours have been described by Kaliaperumal and Umamaheswara Rao (1982). The percentage frequency of tetrasporophytes, carposporophytes and vegetative plants present in the sample was estimated. The length of the erect fronds in different phases of the life cycle of the alga was measured to estimate the mean height of the tetrasporophytes, carposporophytes and vegetative plants and the fronds were divided into 2 groups: group I (<2 cm) and group II (>2 cm), for determining the abundance of these two size classes in the population of *P. heteroplatos*. The number of tetrasporangial sori present in 3 to 5 tufts and cystocarps present on carposporophytes were counted every month and the fresh

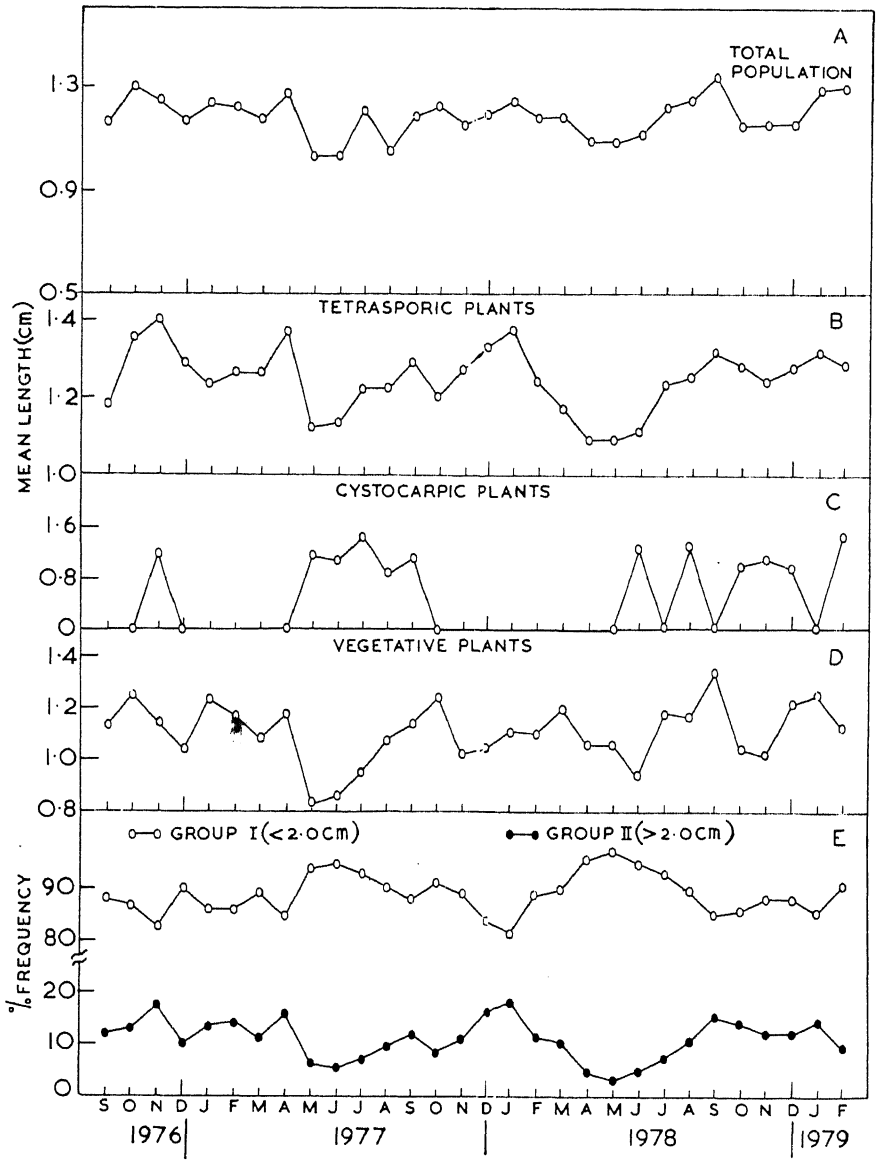


Figure 1. Seasonal changes in length and frequency of two size classes of fronds of *P. heteroplatos*.

weight of the tufts was noted to estimate the seasonal abundance of tetrasporangial sori and cystocarps/g fresh weight of the plant. Methods used for the liberation of spores and estimation of seasonal production of tetraspores and carpospores are described in an earlier communication (Umamaheswara Rao and Kaliaperumal 1983). Eight replicates were used each month for estimating the tetraspore output and data collected on the first day are plotted in figure 2. Since carposporophytes were rarely

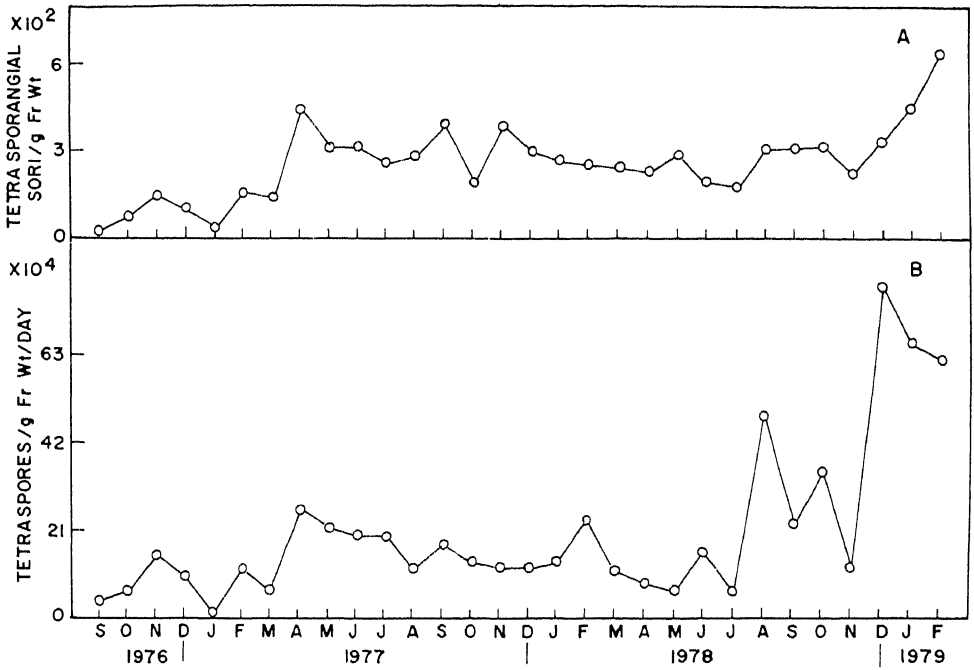


Figure 2. Seasonal variations in the tetrasporangial sori and tetraspore output of *P. heteroplatos*.

found in the population of *P. heteroplatos*, carpospore shedding experiments were conducted in three different months (table 2).

3. Results

3.1 *Growth behaviour*

Monthly changes in frond length and percentage frequency of the two size groups of erect fronds of *P. heteroplatos* are shown in figure 1. Since the data collected at fortnightly intervals from two stations did not vary significantly, mean values obtained for each month ($n = 40-50$) are plotted in figure 1. Though monthly variations in growth were rather irregular in the total population (figure 1A), increase in frond length was seen from May or June and Maximum development of the plants from November to January. The t-value calculated for all the maximum and minimum growth periods of this alga was significant (5·934) at 1 and 5% levels (d.f. = 12), indicating the occurrence of a single peak growth period in a year in *P. heteroplatos*. A single peak growth period can be seen more clearly in the tetrasporic plants (figure 1B) than in the entire population (figure 1A) and vegetative plants (figure 1D). Male plants were not observed in the population analysed and since female plants with cystocarps occurred only for some months, growth data could not be collected separately on this gametophytic phase of the alga.

Figure 1E shows the seasonal fluctuation observed in the abundance of fronds of the

two size classes. Group I fronds occurred in maximum numbers (84·6–96·9 %) during April, May and June and group II fronds from November to January. These seasonal variations in the stature of the plants also suggest that a single peak growth period occurs between November and January in *P. heteroplatos.*

3.2 *Fruiting cycle*

Monthly and yearly mean values of percentage frequency of tetrasporophytes and carposporophytes of *P. heteroplatos* are shown in table 1. From the annual means estimated it is evident that 80·6 % and 19·4 % fruiting and vegetative plants occur in the natural population respectively. Tetrasporophytes were more abundant than carposporophytes and their frequency in different months ranged from 60·5 to 92 %. The yearly mean value calculated for tetrasporophytes was 79·3 %. Monthly averages of carposporophytes given in table 1 show that these plants occur for certain months of the year and the frequency values varied from 0·3 to 7·8 %. The abundance of tetrasporophytes did not vary seasonally.

As shown in figure 2A, there was no seasonal variation in the tetrasporangial sori forming on the plants of *P. heteroplatos* and the number of sori ranged from 187 to 6378/g fresh weight of the plant during the period of study. Cystocarps per gram fresh weight of the plant varied from 766 to 2627 for three different months (table 2).

Table 1. Percentage frequency of tetrasporophytes and carposporophytes in the population of *P. heteroplatos.*

	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Mean
Tetrasporophytes													
1976–77	14·8	63·5	84·0	80·0	42·0	61·0	76·0	79·8	87·0	88·0	76·0	72·3	68·7
1977–78	86·0	88·0	91·2	96·0	88·8	84·0	45·0	75·0	72·0	96·0	88·0	80·0	82·5
1978–79	94·0	76·0	91·0	94·0	88·0	78·0	—	—	—	—	—	—	86·8
Mean	64·9	75·8	88·7	90·0	72·9	74·3	60·5	77·4	79·5	92·0	82·0	76·1	79·3
Carposporophytes													
1976–77	0	0	1·0	0	0	0	0	0	2·0	3·0	5·0	11·5	1·9
1977–78	2·0	0	0	0	0	0	0	0	0	2·0	0	4·0	0·7
1978–79	0	3·0	1·0	1·0	0	2·0	—	—	—	—	—	—	1·2
Mean	0·7	1·0	0·7	0·3	0	0·7	0	0	1·0	2·5	2·5	7·8	1·3

— data not collected

Table 2. Abundance of cystocarps and carpospore output in *P. heteroplatos.*

Month	Cystocarps/g fr. wt.	Carpospores/g fr. wt/day
November 1976	1658	74215
July 1977	766	167040
September 1977	2627	39996

3.3 *Spore shedding*

Figure 2B and table 2 show the tetraspore and carpospore outputs respectively from *P. heteroplatos*. Maximum liberation of both tetraspores and carpospores was seen on the first day and spore output decreased from the second day onwards. Under laboratory conditions, tetraspore output was observed up to 4 days and carpospore output up to 14 days. Maximum shedding of tetraspores was from April to July in 1977 and from August to February in 1978–79 with low values in September and November. In this study, the tetraspore output varied from 1 427 to 7 94 055 spores/g fresh weight of the plant and the carpospore output from 39 996 to 1 67 040 spores (table 2).

4. Discussion

Occurrence of *Pterocladia* in India was reported recently (Umamaheswara Rao and Kaliaperumal 1980) and this is the first ecological study on this genus in India. In the present study on *P. heteroplatos* monthly changes in growth are not marked and maximum development of erect shoots occurs in this alga between November and January. These findings are in agreement with the results obtained by Matsuura (1958), Dixon (1958) and Santelices (1978a) who reported the occurrence of a single peak growth period in *Pterocladia pacifica, P. capillacea* and *P. caerulescens* with maximum development of plants between November and January.

The fruiting behaviour of *P. heteroplatos* was different from other species of *Pterocladia* investigated by Matsuura (1958), Stewart (1968) and Santelices (1978b). These authors observed seasonal variation in the abundance of tetrasporophytes of *P. capillacea* and *P. caerulescens* and carposporophytes of *P. pacifica*. Data presented here (table 1) clearly show that seasonal variations do not occur in the abundance of tetrasporophytes or carposporophytes of *P. heteroplatos* (table 1). However, as observed in *P. capillacea* (Stewart 1968; Oliveira Filho and Sazima 1973) tetrasporophytes were predominant in all months of the year in the natural population of *P. heteroplatos*. Antheridial plants were not found by Santelices (1978b) in *P. caerulescens*. In the present study also male plants were absent in the population of *P. heteroplatos*.

Little is known on the spore producing capacity of *Pterocladia* species. In the present study tetraspore shedding was observed in all months of the year and the spore output was high in *P. heteroplatos* with 7·9 lakhs spores/g fr wt in some months of the year. The values obtained are comparable with the estimates given by Suto (1950) for *Gelidium amansii* and by Kaliaperumal and Umamaheswara Rao (unpublished) for *G. pusillum*. In the occurrence of less marked seasonal growth behaviour, in the absence of seasonality in the fruiting plants and in the production and liberation of tetraspores, *P. heteroplatos* agrees well with *G. pusillum* growing in the same locality (Kaliaperumal and Umamaheswara Rao, unpublished).

Acknowledgements

The authors are thankful to the Head of the Department of Botany, Andhra University, Waltair, for the laboratory facilities. One of the authors (NK) is grateful to

Dr V A Parasuraman for financial help and also to the CSIR, New Delhi, for the award of a fellowship.

References

Boney A D 1965 Aspects of the biology of the seaweeds of economic importance; *Adv. Mar. Biol.* **3** 105–253

Dixon P S 1958 The structure and development of the thallus in British species of *Gelidium* and *Pterocladia; Ann. Bot. London N.S.* **23** 353–368

Kaliaperumal N and Umamaheswara Rao M 1981 Studies on the standing crop and phycocolloid of *Gelidium pusillum* and *Pterocladia heteroplatos; Indian J. Bot.* **4** 91–95

Kaliaperumal N and Umamaheswara Rao M 1982 Seasonal growth and reproduction of *Gelidiopsis variabilis* (Greville) Schmitz; *J. Exp. Mar. Biol. Ecol.* **61** 265–270

Matsuura S 1958 Observations on the annual growth cycle of marine algae on a reef at Mamadzuru on the Pacific coast of Japan; *Bot. Mag. (Tokyo)* **71** 93–109

Oliveira Filho E C De and Sazima I P 1973 Estudos sobre a biologia de algas agrofitas. I. Recolonizacao, brotamento e fenologia em populacoes naturias de *Pterocladia capillacea* (Rhodophyta-Gelidiaceae); *Bol. Zool. Biol. Mar. N.S.* **30** 677–690

Santelices B 1978a Multiple interaction of factors in the distribution of some Hawaiian Gelidiales (Rhodophyta); *Pac. Sci.* **32** 119–147

Santelices B 1978b The morphological variation of *Pterocladia caerulescens* (Gelidiales, Rhodophyta) in Hawaii; *Phycologia* **17** 53–59

Stewart J G 1968 Morphological variation in *Pterocladia pyramidale; J. Phycol.* **4**: 76–84

Suto S 1950 Shedding, floating and fixing of spores of *Gelidium; Bull. Jpn. Soc. Sci. Fish.* **15** 671–673

Umamaheswara Rao M and Kaliaperumal N 1980 Genus *Pterocladia* (Rhodophyceae) from India; *Bull. Bot. Surv. India* **22** 109–111

Umamaheswara Rao M and Kaliaperumal N 1983 Effects of environmental factors on the liberation of spores from some red algae of Visakhapatnam coast; *J. Exp. Mar. Biol. Ecol.* **70** 45–53

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 633-638
© Printed in India.

Seedling anatomy of *Brassica campestris* L

S BABBER and A PILLAI*

Department of Botany, Haryana Agricultural University, Hisar 125004, India

* Department of Botany, University of Rajasthan, Jaipur 302004, India

MS received 6 July 1984; revised 11 February 1985

Abstract. Vascular orientation of one week old seedling of *Brassica campestris* Linn. has been studied. The studies rule out the possibility of splitting and torsion of xylem; instead there is a gradual inward shifting of the xylem differentiation pole. Unlike the existing reports, *Brassica campestris* Linn., in spite of having epigeal germination, shows the continuity of primary vasculature of root-hypocotyl-cotyledon in the first and second foliar node. The cotyledonary node is unilacunar two trace which supports Bailey's suggestion of unilacunar double trace as a basic type.

Keywords. *Brassica campestris*; seedling anatomy.

1. Introduction

Vascularization and orientation patterns of the primary vascular systems of shoot and root in seed plants are of interest both from the developmental and phylogenetic points of view and, therefore, are extensively treated in botanical literature. A good number of publications have appeared on the seedling anatomy during the last two decades. Notable among them are those by Deshpande and Jonaja (1962), Esau (1965), Fahn and Broido (1963), Govil (1972), Pillai and Mehra (1973), Pillai and Kumar (1980) and Kavathekar *et al* (1980). The present investigation is an addition to the literature on this interesting and somewhat controversial subject.

2. Material and method

Seedlings of *Brassica campestris* Linn. were raised in small pots of sand. One week old seedlings were fixed in formalin-acetic alcohol, dehydrated in TBA series, infiltrated with para-TBA and embedded in paraffin. Serial transections (10–15 μm) were stained with safranin and light green combination. Measurements were taken from the radicle towards the plumule. A few foliar nodes above the cotyledonary node were also studied.

3. Observations

The primary root is diarch. The stele is enclosed by a single-layered pericycle and a well-developed endodermis. The xylem forms a central plate flanked by phloem groups. Cambium like cells (about 3·24 mm) appear inner to the phloem patches (figures 2A, B) and the differentiation of cells cut off on the inside into the xylem results in an increase in the volume of the xylem. On the contrary, very few cells are cut off on the outside. Consequent on this increase, the xylem assumes a diamond shape with two

narrower ends at the protoxylem poles. At a distance of 32·4 mm, many small patches of parenchyma cells appear in the xylem core which mark the beginning of the pith. With the development of parenchyma cells, the xylem is cut into small groups (figure 2C). Though the protoxylem elements are still present at their original positions, they appear to gradually disintegrate and a freshly differentiated xylem is observed (figures 2C, D). Later on, the parenchymatous pith becomes increasingly prominent, dividing the xylem core into two groups flanking each protoxylem pole.

At 42·3 mm level, a small group of xylem elements from the root hypocytyl vasculature take up a position between the two protoxylem poles on the side where the first foliage leaf is to develop. This makes a total of five xylem groups (figure 2D). The phloem patches expand laterally followed by splitting at about 46·4 mm level. At a little higher level, six collateral bundles are formed around a well-developed pith, the smallest one being the product of newly differentiating epicotylar procambium (figures 2E, F and 1b). The four larger bundles in pairs flank the erstwhile protoxylem poles and the two smaller ones are lateral.

The pith area increases with the addition of cells. The cells are isodiametric, thin-walled with small intercellular spaces. At successive level, from 49·6 mm, the procambium differentiates on either side of the smaller lateral bundles in the intercotyledonary plane (figure 3G). The two pairs of larger bundles form the cotyledonary traces which start shifting towards the cortex at a level of 51·22 mm. Thus the cotyledonary node is unilacunar two-traced (figures 3A, B). A new procambium develops closing the gaps. At a higher level (51·76 mm), cleavages appear on either side of the axis indicating the formation of cotyledons, which extend around the axis and later on get dissociated completely. The two bundles in each cotyledon maintain their identity in the petiols and give off branches in lamina. The vascular ring gets stretched and assumes an oval shape. The larger of the two bundles, in the inter-cotyledonary plane, becomes the median for the first foliage leaf. On the flanks of this median, two bundles differentiate from the procambium forming the foliar laterals (figures 3B, C). Thus the first foliar node is trilacunar three trace. The median is constituted partly from the root-hypocotyl-cotyledonary-vasculature and the epicotylary procambium,

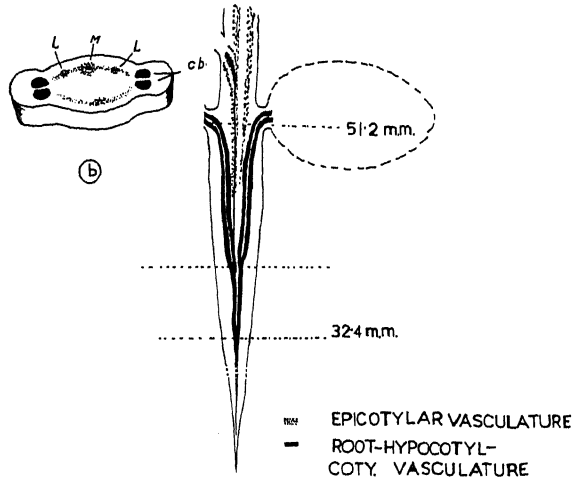


Figure 1. a. Diagrammatic representation of epicotylar and root-hypocotylar cotyledonary

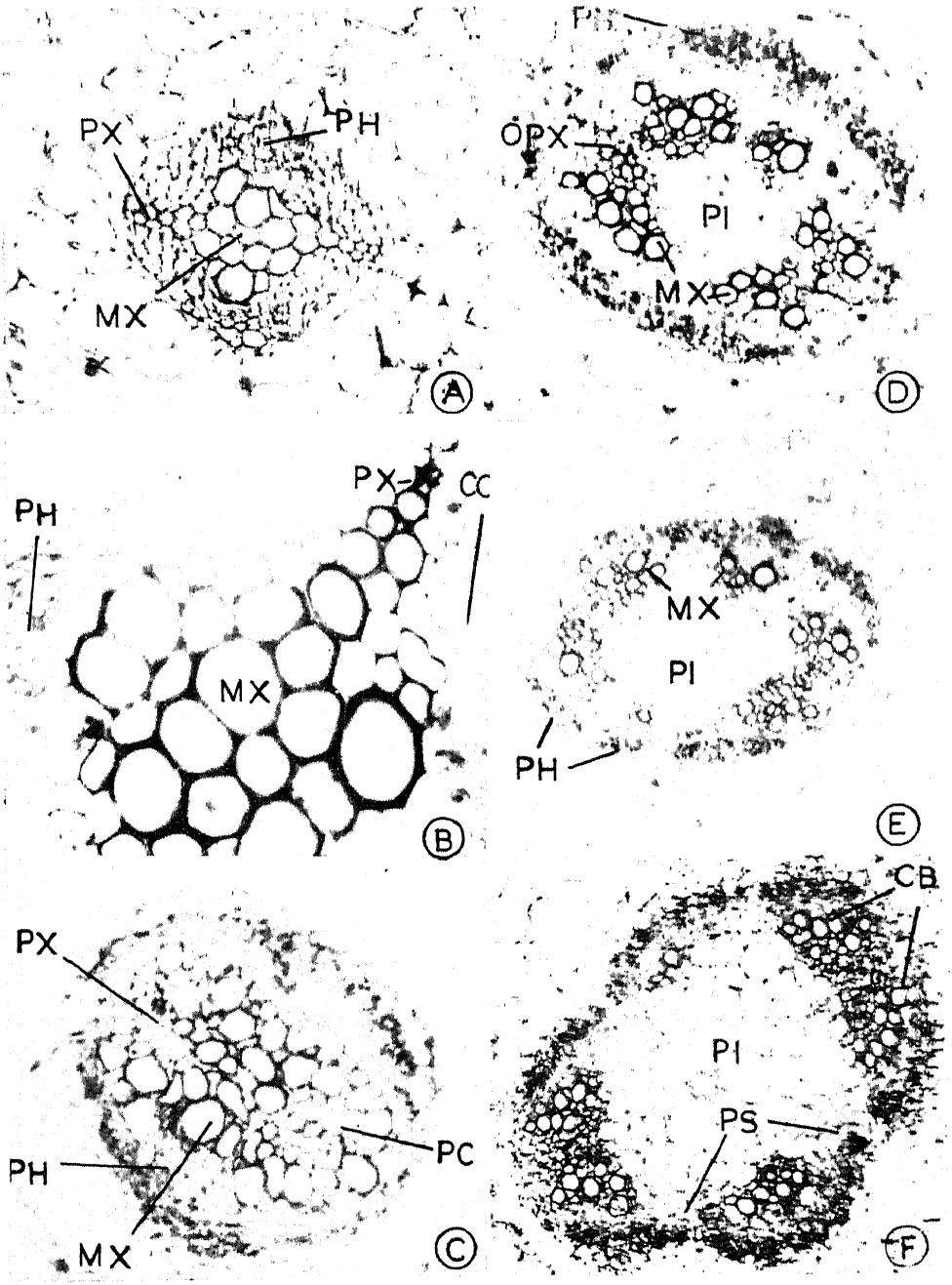


Figure 2. Serial transverse section of the seedling. **A.** Diarch root (× 200). **B.** Initiation of cambium like cells and extension of the xylem core (× 600). **C.** Splitting and differentiation of xylem around protoxylem pole and development of parenchyma cells (× 200). **D.** Formation of five xylem patches and the lateral extension of the phloem and obliterated protoxylem (× 200). **E.** Formation of six bundles with the splitting of the phloem (× 200). **F.** Formation of six collateral bundles (× 200).

Abbreviations: CB – collateral bundle; CC – cambium like cells; MX – metaxylem; OPX – obliterated protoxylem; PC – procambium; PH – phloem; PI – pith; PS – procambium strands; PX – protoxylem; Cb – cotyledonary bundle; FFL – first foliage leaf; L – laterals; M – median.

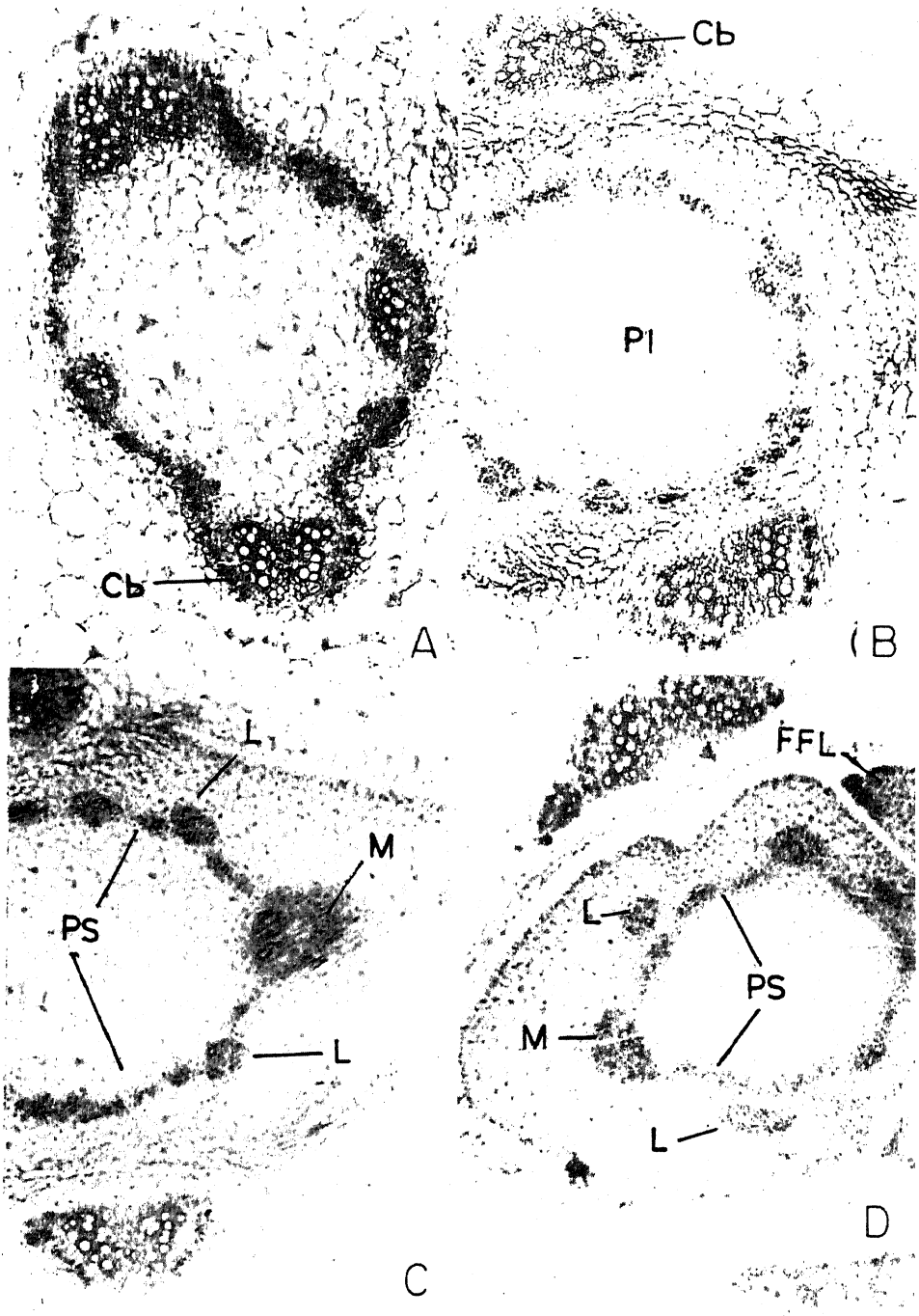


Figure 3. **A.** Departure of the cotyledonary bundles and addition of new vascular elements. **B.** Formation of cotyledons (× 200). **C.** Formation of first foliar node. **D.** Formation of 2nd foliar node (× 200).

whereas, the laterals are entirely from the epicotylary procambium. The vasculature of the second leaf is entirely epicotylar (figure 3D).

Thus the root-hypocotyl-cotyledonary vasculature forms part of the vasculature of the first foliar nodes. The root and shoot vasculature establish connection with each other at the first foliar node.

4. Discussion

The pattern of vascularisation of the seedling and interpretations of the inter-relationship between the vasculature of the root, shoot, cotyledons and epicotyl have engaged the attention of many workers, particularly for the phylogenetic value. In literature, there are many reports of splitting and rotation (torsion) of xylem during transition from the exarch, radial bundles in the root to the endarch, collateral bundles in the shoot or hypocotyl (Eame and MacDaniels 1947; Banerji 1961; Pillai and Sukumaran 1969; Govil 1973). Bonnier (1900), Kavathekar and Pillai (1977), Pillai and Goyal (1979) and Goyal (1981), however, support the concept of the gradual shifting of the pole of xylem differentiation inwards as the vascular elements are traced from root towards the cotyledons. The present studies also support this view, since the protoxylem elements get gradually obliterated *in situ* in the investigated seedling.

A perusal of literature reveals that, in dicots with epigeal germination, the collateral arrangement of the vascular tissue in cotyledons is in direct continuity with the radial pattern in root without any direct connection with epicotyl vasculature as in *Raphanus* (Grassley 1932), *Medicago sativa* (Winter 1932) *Daucus carota* (Esau 1940), in some members of Compositae (Mishra Raj 1970), in some Ranales (Kavathekar and Pillai 1977) and *Ricinus* (Babber 1981). But the present investigation does not seem to agree with this, since *Brassica* has the medians of the first foliar node derived at least partly from the primary vascular system of the root hypocotyl-cotyledon.

Easu (1965) has suggested that this continuity or discontinuity (between root-hypocotyl-cotyledon and epicotyl vasculatures) has been attributed to the very early time of initiation of the leaf primordia; the first leaf traces may be related to the primary tissue of the root and if the primordia are initiated later, the connection is established with the vascular tissue appearing later in development. But the present studies overrule this hypothesis as the leaf primordia are initiated late but with the continuity of root hypocotyl-cotyledon vasculature along the epicotyl vasculature. Thus it indicates that this concept cannot be universally applied.

The studies of cotyledonary nodes suggest that the unilacunar two-trace condition may be phylogenetically significant. Bailey (1956) proposed unilacunar two-trace as the most primitive and added that, even in plants with trilacunar foliar nodes, the cotyledonary node is unilacunar double trace. The occurrence of unilacunar two-trace cotyledonary nodes has also been reported by Kavathekar and Pillai (1977) in some members of Ranunculaceae; Kavathekar *et al* (1980) in Asclepiadaceae, Cruciferae and Pedaliaceae. The claim to primitiveness of the unilacular two-trace node appears to be enhanced by these as well as the present data.

Acknowledgement

The authors are indebted to Prof. S K Pillai for going through the manuscript and to Prof. T M Varghese for facilities.

References

Babber S 1981 *Developmental anatomy of some crop plants.* Ph.D. Thesis, University of Rajasthan, Jaipur

Bailey I W 1956 Nodal anatomy in retrospect; *J. Arnold Arbor.* **37** 269–287

Banerji M L 1961 On the anatomy of teratological seedlings I. *Cosmos bipinnatus* Cav.; *Proc. Indian Acad. Sci.* **B53** 10–19

*Bonnier G 1900 Sur L'Ordre de formation des elements du cylndre central dans la racine et la tige; *C. R. Acad. Sci.* **131** 781–789

Deshpande B D and Jonaja Premlata 1962 Studies in Asclepiadaceae; I. Morphology of *Laptadenia; Pyrotechnica* Dene; *Phyton* **19** 73–84

Eames A J and MacDaniel L H 1947 *An introduction to plant anatomy* 2nd edn (New York: McGraw-Hill)

Esau K 1940 Developmental anatomy of the fleshy storage organ of *Daucus carota; Hilgardia* **13** 175–209

Esau K 1965 *Plant anatomy* 2nd edn (New York: John Wiley)

Fahn A and Broido S 1963 The primary vascularisation of the stem and leaves of the genera *Salsola* and *Suaeda; Phytomorphology* **13** 156–165

Govil C M 1972 Developmental studies in *Argyreia nervosa* Boj. In: *Biology of the Land Plants* (eds) V Puri *et al* (Meerut: Sarita Parkashan) 110–119

Govil C M 1973 Seedling and nodal anatomy of *Nyctanthus arbortristis* L; *J. Indian Bot. Soc.* **52** 113–118

Goyal S C 1981 *Anatomical and morphological studies on some oil-yielding plants.* Ph.D. Thesis, University of Rajasthan, Jaipur

*Grassley F E 1932 *The anatomy of primary body of Raphanus sativus*; M.S. Thesis, University of Chicago

Kavathekar K Y and Pillai A 1977 Studies on the developmental anatomy of Ranales. IV. Seedling anatomy of some members of Ranunculaceae; *Phytomorphology* **27** 240–247

Kavathekar K Y, Goyal S C, Satija S, Jain S and Pillai A 1980 Nodal anatomy in some dicotyledons; In All India Symposium on *Histochemistry, developmental and structural anatomy of angiosperms* (ed.) K Priaswamy, Tiruchirapally 31–39

Misra Raj D N 1970 *Primary vascular differentiation in certain members of the family Compositae.* Ph.D. Thesis, University of Indore, India

Pillai A and Goyal S C 1979 Anatomy of normal and teratological seedling of *Carthamus tinctorius; Phytomorphology* **29** 3–146

Pillai A and Mehra A 1973 Some aspects of the anatomy of *Tecoma stans* (L.) H. B. K; *Proc. Indian Acad. Sci.* **B77** 64–77

Pillai S K and Kumar K B 1980 Seedling anatomy of certain umbellifers. In *Histochemistry, developmental and structural anatomy of angiosperms*; (ed.) K. Priaswamy, Tiruchirapally

Pillai S K and Sukumaran K 1969 Histogenesis, apical meristems and anatomy of *Cyamopsis tetragonoloba; Phytomorphology* **19** 303–312

Winter C W 1932 Vascular systems of young plants of *Medicago sativa; Bot. Gaz.* **94** 152–167

* Original not seen.

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 639-654
© Printed in India.

The veinlet syndrome in the tribe Andromedeae (Ericaceae)

T ANANDA RAO and SWAPNA CHAKRABORTI
Botany Department, Bangalore University, Bangalore 560 056, India

MS received 7 December 1983, revised 2 February 1985

Abstract. Studies on leaves of the genera belonging to the two sub-groups: the *Lyonia* and the *Gaultheria* of the tribe Andromedeae were investigated anatomically. In both the groups there are clear vein ending types and also sclereid idioblasts confined to each taxa. Their morphological features could be regarded as diagnostic criteria of taxonomic value and may also serve as an adjunct character in the relationship of species or groups of species within a genus.

Keywords. Vein endings; sclereid idioblasts; Andromedeae (Ericaceae); taxonomic and diagnostic value.

1. Introduction

The Ericaceae is a family of nearly 100 genera and 5000 species distributed all over the world (Hooker 1876; Drude 1897). Since the last three decades this family has been looked into for a satisfactory classification (Burtt 1948; Watson *et al* 1967; Burtt *et al* 1970; Stevens 1971; Judd 1979, 1981).

According to Niedenzu (1890) sclereid idioblasts occur in a few species of *Diplycosia*, *Gaultheria* and *Pernettya*. In recent years their occurrence has been recorded in a few species of *Rhododendron* (Hayes *et al* 1951), *Leucothoe* and *Craibiodendron* (Rao and Bhattacharaya 1978; Rao and Das 1979a; Rao and Chakraborti 1982). Similarly the different types of vein endings have been recorded in *Leucothoe* (Lems 1964). It is clear from these studies that detailed notings have not been recorded on the types of vein endings and sclereid idioblasts to evaluate their taxonomic range. An attempt has been made in that direction in the tribe Andromedeae, and some pertinent taxonomic questions discussed.

2. Material and methods

The materials for this study were through the courtesy of the Directors/Curators of several herbaria, here abbreviated following the symbols published by Holmgren *et al* (1981).

The clearing technique is after Rao and Naidu (1981) and the categorisation of the types of vein endings, sclereid and tracheoid idioblasts after Lems (1964), Rao and Bhupal (1973) and Rao and Das (1979b) respectively.

3. *Leucothoe* D. Don

Leucothoe sensu lato is considered as an assemblage of different taxa distributed in several geographical areas, chiefly Brazil, North America, S. E. Asia extending up to Japan.

3.1 *Materials*

L. acuminata (Ait.) G. Don (*Andromeda acuminata* Ait. var. *europe*), Andri Walsteri, *s.n.* (CAL); USA, Florida, Jacksonville, A. H. Curtis 4354 (DS). *L. angustissima* (Thnb.) Sleum., S. America-Minas Gerais, G. Matschlench 31564 (L). *L. andina* Sleum., Peru, Friedberg 685 (L). *L. axillaris* (Lam) DC. (*Andromeda axillaris* Lam., Syn. *A. catesbaei* (Walt.), Europe, *s.l., s.n.* (CAL); Medison country, I. F. Logue 9081 (CAL); USA, Red Springs, Carolina, P. O. Schallert *s.n.* (DS); S. America Herb Persoon *s.n.* (L); R. K. Godfrey 3727 (US). *L. boliviensis* sleum., Bolivia, Rincon de la Victoria, Tarija, Turps, Logname, Lopez 5132 (DS); Bolivia, Herzog 1634a (L). *L. bracamorensis* DC., Europe *s.l.s.n.* (CAL). *L. bradei* Sleum., Brazil, Duarte 8927 (L). *L. buxifolia* DC., Grown in R. B.G. Kew and also in Paris, *s.l.* 1840 (DS). *L. catesbaei* (Walt.) A. Gray (syn. *L. fontanesiana* (Steud.) Sleum.), USA, N. Carolina, Bittmore, A. Eston 1280 (CAL); USA, N. Carolina, Blue Ridge, Le Roy *et* Rugar *s.n.* (GAS). *L. chapaensis* Kinoshita-Couves, Brazil, Irwin *et al.* 12862 (L). *L. chlorantha* (G. Don) Gray *et* DC., Japan, Hakodate, Maximowicz *s.n* (CAL). *L. chlorantha* (Cham.) DC. var. *Chlorantha,* Brazil, Hatschbach 22815 (L). *L. coriifolia* (Billb.) DC., Brazil, RDJ garden, *s. l., s.n.*, (CAL). *L. davisiae* Torr. ex A. Gray, USA, California, Ottoway creek, H. L. Hason 12526 (GAS). *L. duartii* Sleum., Brazil, Hatschbach 35379 (L). *L. editorum* Fern. *et* Schub., USA, N. Carolina, C. R. Bell *s.n.* (CAL). *L. elongata* Small, USA, Georgia, P. S. Schallert *s.n.* (DS). *L. eucalyptoides* (Ch. et Schl.) DC., S. America, *s.l., s.n.* (CAL); Brazil, Porto Alegre, B. Rems 40072 (DS); Brazil, Capell 20 (L). *L. fontanesiana* (Steud.) Sleum., Cult. Netherlands, Roon 26851 (L); W. Duncan 8081 (US), *L. grayana* Marin., Japan, Nambu, Maxim owicz *s.n.* (CAL) *L. grayana* Maxim. var. *nervosa* Nakai, Japan, Herb. d' Alleinett *s.n.* (L). *L. griffithiana* C. B. Clarke, E. Himalaya, Griffith 3485 (CAL). *L. hispidula* Meisn., Brazil, Duarte 8098 (L) *L. intermedia* Meisn., Brazil, Hunt 6449 (L). *L. Keiskii* Maxim., Japan, Tokyo, Hayakowa 1795 (CAL); Japan, Makino Herb. 81601 (L). *L. mariana* DC., USA, Florida, *s. l., s.n.*, (CAL). *L. mariana* (Hemsl.) Small, Maxico, Ynez Mexia 9157 (GAS). *L. mexicana* (Hemsl.) Small, var. *mexicana* Hemsl., Salvador. Weberling 285 (L). *L. multiflora* DC. var. *petiolaris* Meisn., Brazil, L. C. Williams, 7503 (DS). *L. niederleinii* Sleum., Brazil, Rambo 30902 (GAS); Brazil, Rambo 32752 (L.) *L. oleifolia* (Cham.) DC., Brazil, Garden *s.l. s.n.*(CAL); Rio de Janeiro, A. C. Borade 11108 (GAS); Widgren 326 (US). *L. oleifolia* (Cham.) DC. var *hispidula* (Meisn.) Sleum., Brazil, Hatschbach 27511 (L). *L. organensis* Gardn., Brazil, Glaziou 3736 (L). *L. pinetorum* Standl. & Williams, Maxico, Valle de Guards, Jalisoo, Luz Ms. V de Puga *s.n.* (DS). *L. pohlii* (G. Don) Sleum., Brazil, Hatschbach 30123 (L). *L. populifolia* (Lam.) Dippel, cult., W. Carolina, F. G. Mayor & P. M. Mazzee 10791 (GAS). *L. pulchella* DC. var *pulchella* DC., Brazil, Hatschbach 17380 (L). *L. racemosa* A. Gray, America, L. S. Beard 665 (CAL); Maryland, V. Coville, *s.n.* (CAL); Washington, F. L. J. Boettcher 15 (DS). *L. recurva* (Bucki.) A. Gray, North Carolina, Pilot Mt., *s. l.* (DS) *L. revoluta* (Spr.). DC, Brazil, Magathes 17459 (L). *L. salicifolia* DC., Mauritius Isl., Murray *s.n.* (CAL); Insula Borbonia, *s.l., s.n.* (DS). *L. serrulata* DC. (syn. *L. revoluta*), Rio de Janeiro, Guilleum *s.n.* (DS). *L. uleana* Sleum., Brazil, Duarte 8482 (L). L. sp. Brazil, A. F. Regnell 179 (CAL); 855 (CAL); 186 (CAL).

3.2 *Observations*

A study of the cleared laminae, hand sections and macerations revealed that the vein endings display diverse features of morphological interest. They can be grouped under

different lines of differentiation. In the first case, the vein endings exhibit sharply pointed tracheidal veins. In the second, the curved vein endings have tracheoids of varied shape and size aggregates. In the third, the vein endings are thick and short with a cluster of small tracheoids. Finally, they have enlarged terminal or sub-terminal translucent cells. These cells have a thin wall, free from pits, and rarely with contents.

3.3 *Typology*

3.3a *Conventional tracheids*: They are characterised by simple vein endings without sheathing cells. They exhibit a few variations of minor importance such as the presence of a few brachytracheoids at the vein endings. This is exemplified in *L. recurva* and *L. racemosa.*

3.3b *Curved and bulbous veinlets with a few brachytracheoids*: The curved and clavate feature of the veinendings is normal though not unique for the following species. *L. axillaris* (figure 8); *L. boliviensis, L. buxifolia, L. chapaensis, L. editorum* (figures 15, 16) *L. frontanesiana* and *L. keiskii.*

3.3c *Vein endings with a few translucent cells:* This feature is exemplified in *L. andina, L. bracamorensis* (figures 1, 2, 17, 23, 24) *L. duartii, L. hispidula, L. mexicana* var. *mexicana, L. niederleinii, L. nummularia. L. pohlii, L. racemosa* and *L. salicifolia* (figures 4, 5). Among the above mentioned species, leaves of *L. bracamorensis* possess conspicuous more or less translucent cells at the vein endings (figures 23, 24). This situation is comparable with the vein endings of species of *Craibiodendron* mentioned elsewhere in this paper.

3.3d *Vein endings with relatively short and small sized brachytracheoids:* They are exemplified in *L. angustissima, L. bradei, L. chapaensis, L. crassifolia, L. eucalyptoides* (figure 3), *L. organensis, L. pulchella* var. *pulchella.*

3.3e *Vein endings with fibrous cells protruding into the mesophyll*: They are observed in *L. elongata*; *L. grayana* var. *pruinosa L. grayana* var. *nervosa, L. grayana* var. *oblongifolia, L. populifolia* f. *psilocarne, L. multiflora* var. *petiolaris, L. nummularifolia* and *L. pinetorum.*

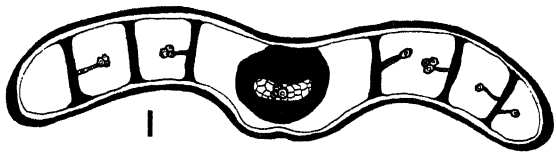
3.3f *Veins with splayed appearance*: They are observed in the leaves of *L. chlorantha* var. *chlorantha* and *L. serrulata.*

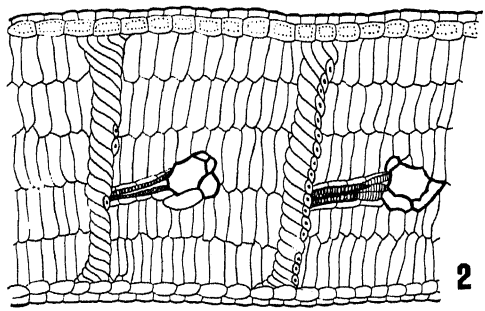
This striking pattern of vein disposition is normal though not unique for the above two taxa in this genus.

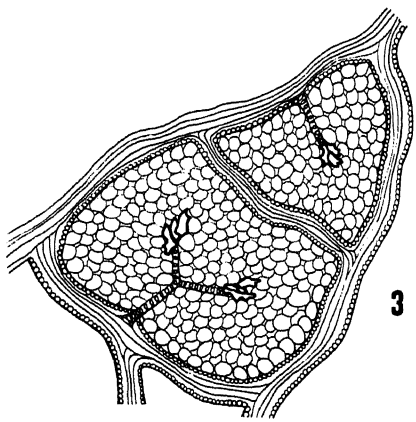
3.3g *Veins sheathed*: With relatively thick-walled cells and a few of them clustering at the vein endings, have been observed in *L. acuminata, L. davisiae* and *L. oleifolia* (figure 18).

4. *Craibiodendron* W. W. Sm.

The genus was first described by W W Smith (1911) as an honour to Mr W G Craib, on the basis of Burma and Siam specimens of *C. shanicum.* deposited in Kew and





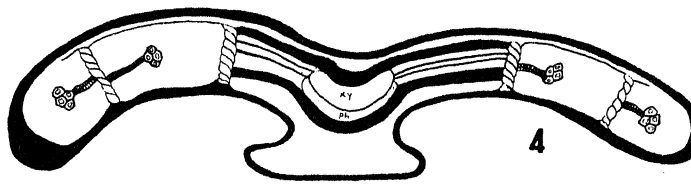


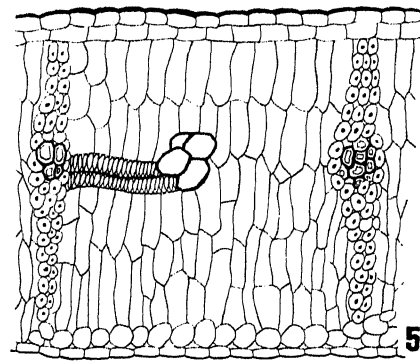
Figures 1–3. *Leucothoe bracamorensis* DC. **1.** Stylised diagram of transectional view, (× 240), **2.** TS of the leaf sector showing the cluster of translucent cells at the vein endings (× 225). **3.** *L. eucalyptoides* (Cham. et Schl.) DC. Brechytracheoids at the vein endings (× 125).

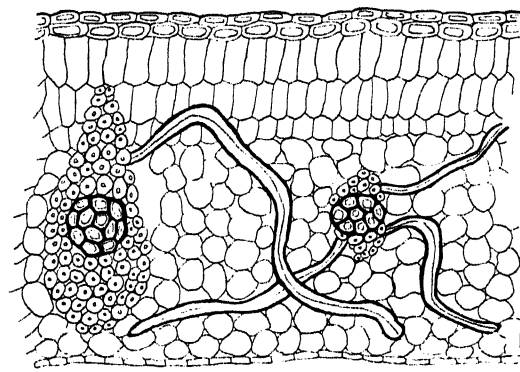
Edinburgh herbaria. The six species of this genus are confined to South-East Asian region, namely India, China, Burma, Cambodia and Thailand.

4.1 *Materials*

C. bidupense Cogns., Indo-china, Annam, Poilane 30731, ex Paris (K). C. *forrestii* W. W. Sm. (Syn. *Quercus semecarpifolia* (Sm.), non. loc., Forrest 1143 (E). *C.henryi* W. W. Sm., Burma, C. E. Parkinson 4985 (CAL); East Asia, China, Explorations of George Forrest 1917 – 1919; 18391 (CAL); Non loc., Henry 13137 (E). *C. kwangtungense* S. Y. Hu, China Kwangtung, Ting-Wu-Shan, Y. Tsieng 792 (A): Kwangsi, Sch-feng Dar Shan, S. Nannong 2000 ft, Ching 8293 (E). *C. kwangtungense* var. *frutescens* Hu, China, Kwangsi, Shang-sze, Shap-man-tawi, Shan, W. T. Tseng 22252 (A), *C. mannii*

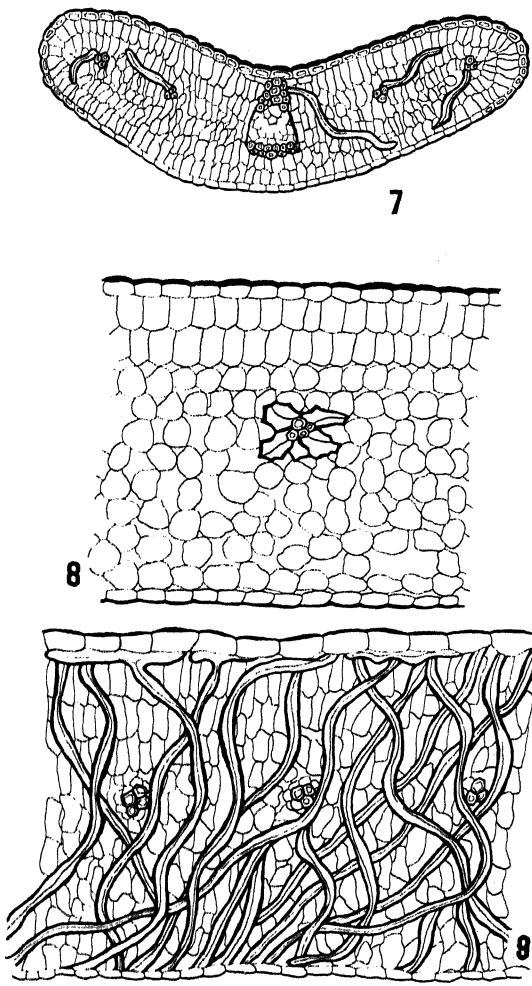






Figures 4–6. *Leucothoe salicifolia* DC. (Murphy *s.n.* CAL) **4.** Stylised sketch showing the varied mesophyll features (× 225). **5.** TS showing enlarged cells in aggregates at the vein endings (× 400). **6.** *Pernettya punctata* Klotzsch. Idiofibrosclereids in the vicinity of vascular bundles (× 225).

W. W. Sm. (–*Leucothoe mannii* King *et* Prain in MS. –), India, Nagaland, Naga Hills, N. L. Bor 2828 (ASSAM); India, Jowai, Dr. King's collector *s.n.* (CAL); Jaintea Hills, Dr King's collector *s.n.* (CAL); Juudmaka Pahar, C. Mann *s.n.* (CAL) Naga Hills, N. L. Bor 2828, 6271 (DD). *C. shanicum* W. W. Sm., India, Manipur, Shugnu, D. B. Deb 2633 (CAL); Indo-China, Siam, Chiengmai, A. F. G. Kerr 1288a Ex Herb. Kew (CAL); Upper Burma, Southern Shan State, Taungyi, A. Khalil *s.n.* (CAL); Maymyo, A. Khan 117 (CAL); Maymyo near Ye. Chan, O., Maung BA PE 13178 (CAL): Palse, Chlmi Hill, Manilue, 4000 ft, S. Mokim 1171 (CAL); non loc., J. C. Prazer 123 (CAL), Kaupetlet, P. H. Tracta, C. B. Smales 134 (CAL); Taungyi, S. Shan States, H. W. A. Watson 234 (CAL);

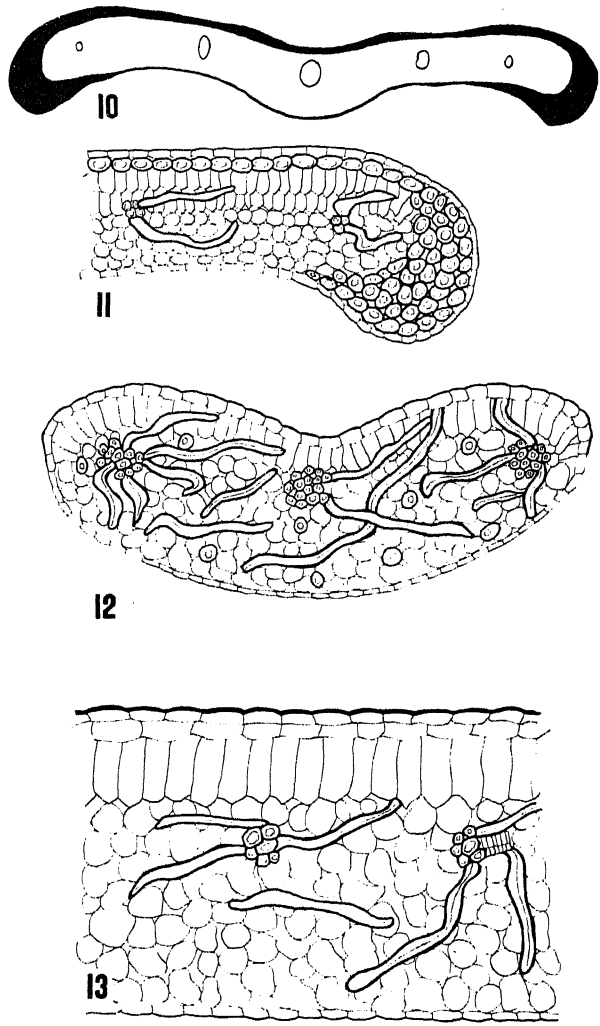


Figures 7–9. (× **225**). TS of leaf sectors: **7.** *D. pinifolia* Stapf (G. D. Haviland 1208, CAL) idiofibrosclereids around the vein bundles. **8.** *Leucothoe axillaris* (Lam) DC. enlarged cells surrounding the vein. **9.** *Diplycosia microphylla* Bece (Griffith 3464, CAL)—filiform sclereids; Note the criss-cross disposition in the mesophyll.

Taungyi, S. Shan States, *s.l., s.n.* (CAL). *C. yunnanense* W. W. Sm., East Asia China, Yunnan, Expl. of G. Forrest, 1917–1919 No. 15939 (CAL); Upper Burma, Forest 1715 (K); Indo-China, Annam, M. Poilane 30927 (L). *C. sp.* Yunnan, Kiukiang valley; T. T. Yu 21046 (E).

4.2 *Observations*

A study of the cleared laminae, hand sections and macerations revealed that the vein endings display spheroidal or sub-spheroidal or polyhedral to oblong-shaped inflated cells. This feature appears to be a generic feature, though the cell vary slightly in shape and size from species to species. They are observed singly or in pairs, and sometimes in



Figures 10–13. TS of leaf sectors. **10.** *Pernettya ciliaris* D. Don ex G. Don (C. G. Pringle 4115, CAL) diagrammatic outline of the structural features, (× 25). **11.** *P. ciliaris* D. Don ex. G. Don (C. G. Pringle 4115, CAL) idiofibrosclereids and marginal strand, (× 225). **12.** *Diplycosia pinifolia* Stapf. and **13.** *Gaultheria nummularioides* D. Don.— idiofibrosclereids in the vicinity of veins (× 225).

threesome. The cells do not have pitted or annularly-thickened walls. On staining with safranin the walls appear to react like tracheary elements, indicating that something more than the primary cell-wall is present. The cell-walls are relatively thick, weakly birefringent in polarised light in contrast to those of tracheary elements. These cells have colourless contents and infrequently one could see unspecified brown contents, apparently resembling tannin. The precise nature of the contents was not determined (figures 19–22, 25, 26).

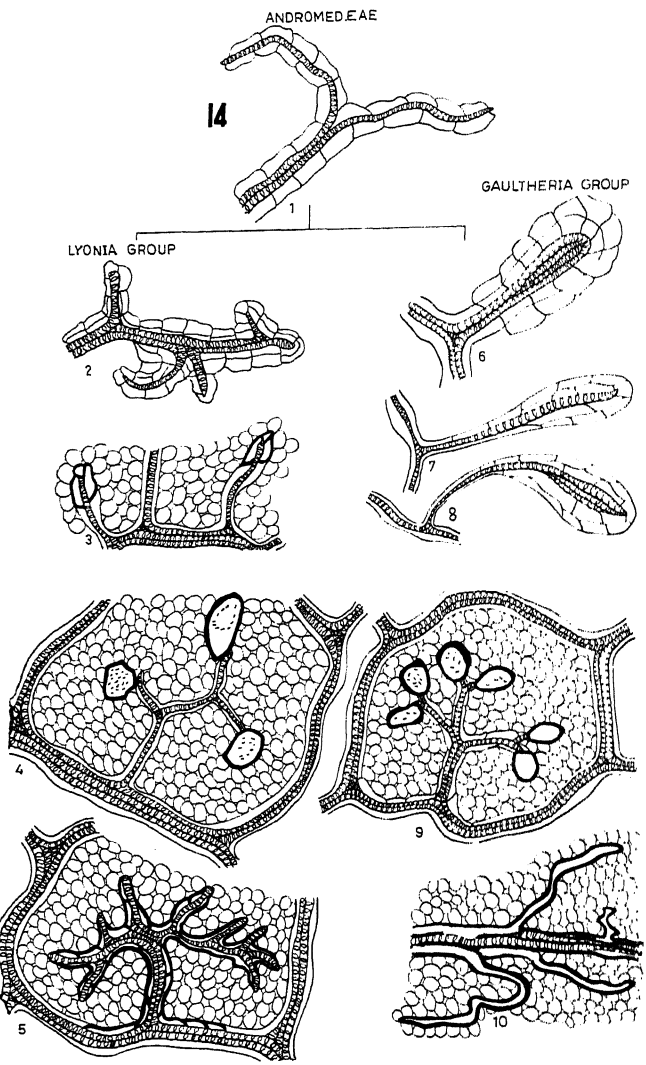
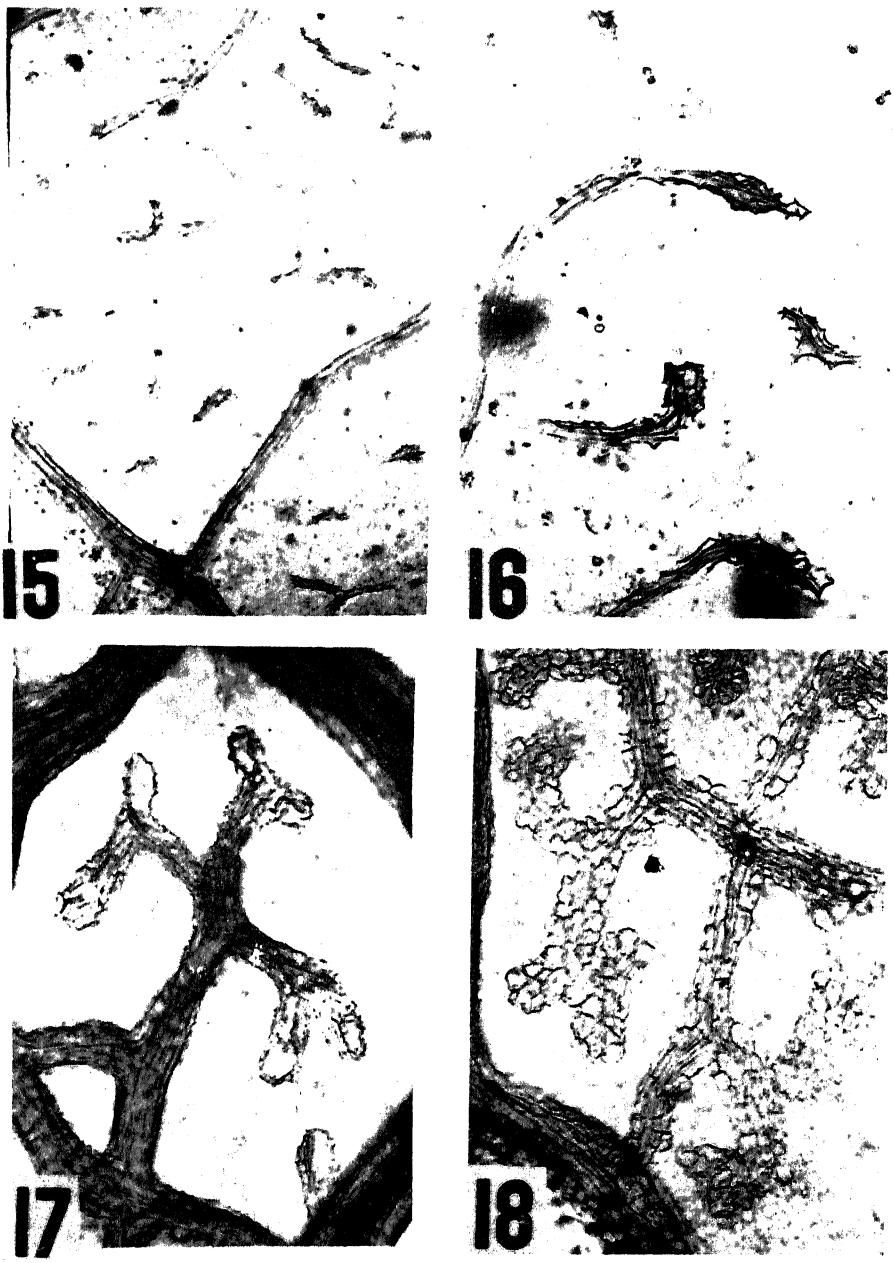
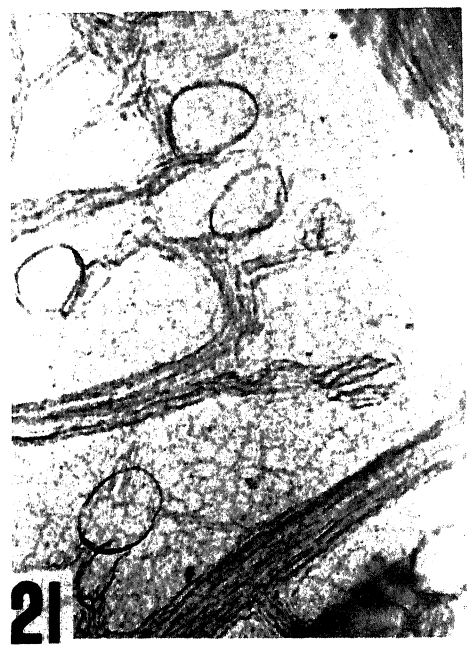


Figure 14. Morphological trends of vein endings in the two subgroups of *Andromedea sensu* Judd (1979)—(semi-diagrammatic sketches, (× 225).
Lyonia group. **1.** Conventional tracheids—*Andromeda*. **2.** Stout and thick vein endings wit sheathing cells—*Agauria*. **3.** Sheathing cells confined to vein ends. **4.** Terminal or sub terminal inflated spheroidal or sub-spheroidal cells—*Craibiodendron*. **5.** Splayed appearanc of vein endings—*Lyonia*.
Gaultheria group **6.** Sheathing cells in multiples at the vein endings *Zenobia*. **7** and **8.** Curve or straight bulbous vein endings—*Leucothoe*. **9.** Terminal or sub-terminal inflated cells at th vein endings—Leucothoe bracamorensis. **10.** Sclerenchymatous fibres traversing int adjacent mesophyll tissue—*Gaultheria*.

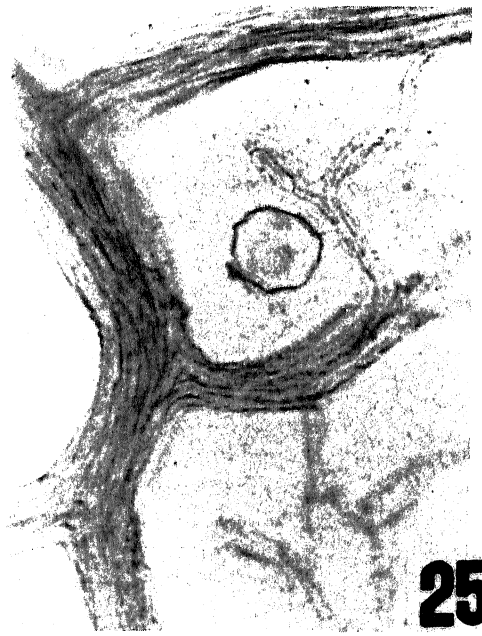
In all probability they could be considered as terminal inflated parenchymatou cells. This is a distinct feature of all the six species investigated. In this respect this genu is distinct from all other genera especially in the vein endings.

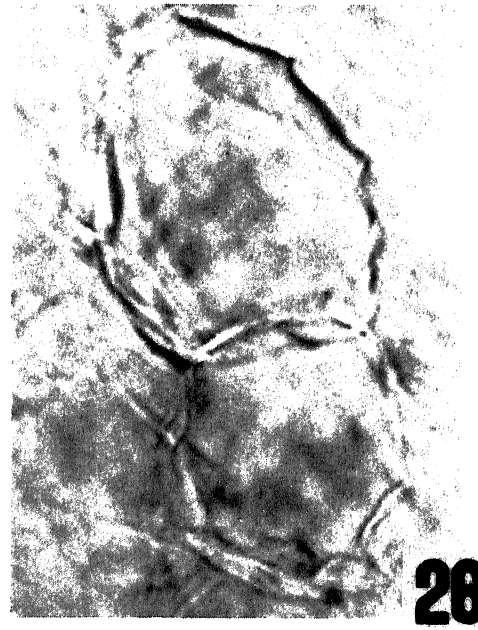


Figures 15–18. Photomicrographs of cleared leaf sectors of: **15** and **16.** *Leucothoe editorum* Fern. et Schub. (C. Ritchis Bell *s.n.* CAL) showing terminal straight or curved bulbous vein endings (× 60 and × 120). **17.** *L. bracamorensis* (*s.l., s.n.*, CAL) translucent spheroidal to oval terminal parenchymatous cells (× 120). **18.** *L. oleifolia* (Cham) DC. (*s.l., s.n.*, CAL) sheathed cells around vein endings and also veins (× 120).



Figures 19–22. Photomicrographs of cleared leaf sectors of: **19.** *Craibiodendron mannii* W. W. Sm. (N. L. Bor 2828, ASSAM) Terminal inflated spheroidal, oblong or polygonal cells (× 240). **20.** *C. manni*: W. W. Sm. (Dr. King's collector *s.n.* CAL) a pair of sub-terminal inflated cells (× 240). **21, 22.** *C. shanicum* W. W. Sm. (D. B. Deb 2653, CAL). Terminal inflated cells, non-inflated vein endings and also inflated vein endings; **22.** Vein endings with two slightly inflated cells holding a polygonal cell, each (× 240).





Figures 23–26. Photomicrographs of cleared leaf sectors of: **23, 24.** *Leucothoe bracemorensis* DC. (*s.l., s. n.,* CAL) showing oppositely oriented terminal cells at the vein endings (× 120). **24.** Splayed vein type holding terminal inflated cells (× 240). **25, 26.** *Craibiodendron mannii* W. W. Sm., **25.** Sub-terminal polygonal inflated cell with contents of unspecified nature (× 240). **26.** Enlargement of terminal inflated cells. Note the wavy outer border of cell-wall, (× 480).

5. *Pernettya* Gaudich

This genus consists of 20 species distributed in Tasmania, New Zealand and South America (Probably to be united with *Gaultheria* L.).

5.1 *Materials*

P. angustata Benth., North America, I. F. Halton 625 (CAL). *P. ciliaris* D. Don ex G. Don, Michoacan State, C. C. Pringle 4115 (CAL). *P. ilicifolia* Miq. Valdivia, Dr. Otto Buchtin, 255635 (CAL); Chile, *s. l.* 544 (CAL). *P. macrostigma* Col., New Zealand, North island, Central Plateau, A. M. Orchard 3339 (AM). *P. microphylla* Gaudich, ex G. Don, South America, D. C. Ranes 730 (CAL); *P. mucronata* Gaudich. Ex. G. Don, South America, *s.l.*, 958 (CAL); South America, Chile, *s.l.*, 817 (835) (CAL); Hermit Island, Capehorn, Antactic exp., J. D. Hook, *s.n.* (CAL) *P. nana* Col., New Zealand, Lake Rukakt, P. Hynes *s.n.* (AK). P. *pentlandii* DC., Andibus Quensibus, K. Spruce 5028 (CAL). *P. punctata* Klotzsch, Valdivia, W. R. F. Hohenecker 867 (CAL), *P. tasmaniea* Hook. f., Tasmania, R. C. Gunn 402 (CAL).

5.2 *Observations*

A feature of morphological interest in all the above ten species is the presence of strand of sclerenchyma around the major and minor veins. Further, the frequent occurrence of strand fibres traversing the adjacent tissue gives the appearance of idioblastic sclereids in transections. These idiofibrosclereids are frequently observed in the leaves of *P. tasmanica* Hook. f., *P. punctata* Klotzsch (figure 6) and *P. ciliaris* D. Don ex. G Don (figures 10, 11).

6. *Gaultheria* Kalm ex L.

This genus consists of more than 200 species and is chiefly circum-pacific in distribution. Only eight out of twelve Indian species were available for study.

6.1 *Materials*

G. fragrantissima Wall., Kerala, A. Meebold 13347 (CAL); Jalapahar, C. W. Cousins 200 (CAL); Kursiang, King's Collector 213 (CAL); Tamil Nadu, C. E. C. Fisher 2531 (CAL); Manipur, George Watt 6873 (CAL); Shillong, H. Deka 18302 (CAL); Kemang, R. S. Rao 7689 (CAL); Wallich 765 (CAL); Nepal, M. L. Banerji 68 (CAL). *G. griffithiana* Wight, Darjeeling, Jalaphar, J. B. Gamble 8197 (CAL); Sikkim, T. C. White 188 (CAL); Assam, Jpvo, Reporter, Govt. of India 11435 (CAL); Naga hills, George Watt 6885 (CAL); Nefa, R. S. Rao 10477 (CAL). *G. hookeri* C. B. Cl., Sikkim, Teng, Risu and Rhomoo 3107 (CAL). *G. leschenaultii* DC., Nilgiris, *S.L.* 113 (CAL). *G. nummularioides* D. Don, Wallich 1524 (CAL); Nepal, V. Puri 531 (CAL); Assam, R. S. Rao 1280 (CAL); Sikkim, Telung hills, *s.l.* 360 (CAL); W. Himal., Kumson, Innyet 24643 (CAL); Kumaon, H. Collett 176 (CAL); Meghalaya, Cheerapunji, G. Panigrahi 4796 (CAL). *G. pyrolaefolia* Hook. F. ex C. B. Cl. Darjeeling, South Phulut, I. H. Burkill 25250 (CAL); E. Himalaya, S. N. Mitra 9620 (CAL); Sikkim, S. N. Mitra 238 (CAL): East Nepal M. L. Banerji 809 (CAL). *G.*

repens Blume, Nepal, J. Scully 235 (CAL); Darjeeling, J. S. Gamble 7448 (CAL); Manipur, Chingsow, George Watt, 8700 (CAL). *G. trichophylla* Royle, Pakistan, Hazara Dist., Inayeti Hossain 19900 (CAL); N. W. Himalaya, Garhwal, J. F. Duthie 14524 (CAL); Assam Naga Hills, R. S. Rao 1636 (CAL); Kashmir, B. M. Wadhwa and J. N. Vohra 612 (CAL); E. Himalaya, Karponang Change, K. Biswas 7168 (CAL); Darjeeling, Sandakph to Phulut, B. Safui 1757 (CAL); Naga Hills, H. Collett 119 (CAL); Shillong, I. H. Lace 328 (CAL); Sikkim Himalaya, Jamfri, G. Q. Gammiee 192 (CAL); Chamba I. H. Lace 14600 (CAL); W. Himalaya, Garhwal, M. A. Rau 101188 (CAL).

6.2 *Observations*

Sclereids are not present in the following species:

G. griffithiana, G. hookeri, G. leschenaultii and *G. pyrolaefolia* Idiofibrosclereids are present in *G. nummularioides* (figure 13) *G. repens* and *G. trichophylla.* They are conspicuous in the leaves and often have wavy outlines.

Terminal sclereids and tracheoids are present in *G. fragrantissima.* Sclereids are filiform whereas tracheoids are varied in shape and size.

7. *Diplycosia* Blume

This genus consists of 60 species chiefly distributed in Malaysia. Only 11 species were available for study.

7.1 *Materials*

D. buhomid (A. Gray) Merr., Philippines, Luzon, Mt. Santo Tomas, A. D. E. Elmer 5932 (CAL). *D. calelanensis* Elmer, Philippines, A.D.E. Elmer 14262 (CAL); Borneo, G. D. Haviland 1135 (CAL). *D. erythrina* King and Gamble, Java, G. King 843 (CAL); Perak, L. Wray 3816 (CAL); Sumatra, H. O. Forbes 20529 (CAL); *D. glabra* Merr., Philippines, J. K. Santos 31784 (CAL). *D. heterophylla* Blume, Java, Hooker 273 (CAL); Perak, L. Wray 364 (CAL); Sumatra, H. O. Forbes 1049 (CAL). *D. kinabaluensis* Stapf, Borneo, G. D. Haviland 1086 (CAL). *D. lancifolia* Ridley, Malay Peninsula, State of Pahang, W. D. Barnes 10891 (CAL). *D. lalifolia* Blume, Malay Peninsula, Perak, King 6797 (CAL). *D. microphylla* Becc., Malaya, Griffith 3464 (CAL). *D. pinifolia* Stapf, Borneo, G. D. Haviland 1297 (CAL). *D. trinervia* Elm., Philippines, A. D. E. Elmer 13800 (CAL).

7.2 *Observations*

The following details are recorded:

Filiform sclereids branching or not branching, dispersed throughout the mesophyll in dense or loose strands observed in *D. buhomid, D. calelanensis, D. glabra, D. heterephylla, D. lancifolia, D. latifolia, D. microphylla, D. pinifolia* (figures 7, 9, 12) and *D. trinervia.*

Tubuliform sclereids at the vein endings are observed in *D. kinabaluensis.* These sclereids have thin cell-wall, wide lumen and unequal width.

Spheroidal and sclerotracheoids at the vein endings have been observed in *D. erythrina*. It is clear from the specimens sampled that they have sclereids of varied types. The types of sclereids, however, in *D. erythrina* and *D. kinabaluensis* may prove to be of diagnostic interest at specific level.

8. Conclusions

8.1 *Morphology*

The taxonomic importance of vein endings at the family level in angiosperms is very doubtful (Strain 1933). However, at the generic level in certain cases, it may be of diagnostic interest (Lems, 1964). In the present studies on the five genera: *Leucothoe, Craibiodendron, Gaultheria, Diplycosia* and *Pernettya,* there are many species or groups of species showing characteristic dissimilar or similar veinlet features. In *Leocothoe* it is revealed that there are 5 types of vein endings represented by a single or groups of species within the genus. In *Craibiodendron* the terminal inflated parenchymatous idioblasts at the vein endings is of diagnostic value for the genus. In *Gaultheria* there are three groups of species. In the first group, the vein ends are simple and quite unlike those of other genera. In the second group, the idiofibrosclereids accompanying the veins extend beyond the vein ends and make inroads into the adjacent tissue. In the third case, sclereids and tracheoids occur side by side. Without information about many more species from the genus it is difficult to comment on the general significance of leaf sclereids except that they are more likely to be of diagnostic value for a species or groups of species of this genus. In *Diplycosia* it is evident that the majority of species have filiform sclereids. However, in a few species one could observe the co-occurence of sclereids and tracheoids at the vein ending. Finally, in *Pernettya* the sclerenchymatous fibres accompanying the veins make an inroad into the adjacent mesophyll. This feature is found in all the investigated species of this genus. Clearly more information is required on the other species before commenting on the significance of this feature.

8.2 *Taxonomic Value*

The present study has demonstrated that in the general *Leucothoe, Gaultheria, Diplycosia, Craibiodendron* and *Pernettya* certain anatomical features could be regarded as diagnostic criteria of taxonomic value and may serve as an adjunct taxonomical feature in the relationship of species or groups of species within a genus. Niedenzu (1890) has utilised spicular cells as systematic criteria in establishing the tribe *Gaultherieae* represented by *Gaultheria, Diplycosia* and *Pernettya*. Stevens (1970, 1971) of the opinion that Niedenzu's grouping of a few genera under *Gaultherieae* is arbitrary and cannot be recognised at the tribal level. Further, he has included under the tribe *Andromedeae* (*pro parte*) *Andromeda* L., *Oxydendrum* Dc., *Craibiodendron* W. W. Sm. (Including *Nuihonia* Dop), *Pieris* D. Don, *Aquaria* (DC.) Hook. fil., *Agarista* D. Don. *Arcterica* Coville, *Chamaedaphne* Moench, *Zenobia* D. Don, *Leucothoe* D.Don, *Gaultheria* L. (including *Chiogenes* Salisb. ex. Tbrr. and *Pernettya* Gaud.), *Pernettyopsis* King *et* Gamble, *Tepuia* Camp and *Diplycosia* Bl. Of these *Andromeda, Oxydendrum* and *Chamaedaphne* are considered to be isolated genera while the remaining taxa fall into two general groups that are referred to by Stevens (1970), and

here as *Lyonia* group and the *Gaultheria* group (Judd 1979). Stevens (1970) included *Lyonia, Craibiodendron, Pieris, Arcterica, Agauria* and *Agarista* under one group, *Lyonia,* and in the *Gaultheria* group, *Leucothoe, Zenobia, Gaultheria, Pernettyopsis, Diplycosia* and *Tepuia* were included.

On the basis of vein ending types, *Andromeda, Oxydendrum* and *Chamaedaphne* belong to simple thin pointed types (Lems 1964). Such tapering tracheids are probably a primitive feature. However in the two sub-groups of the Andromedeae recognised by Judd (1979) there is a clear morphological trend characteristic of many members of each genus (figures 14 (1–10)). These trends in ericalean lamina appear to be very common (Bocher 1981). In the *Lyonia* group, certain modifications of vein endings characteristic of each genus are observed. Firstly, starting from the simple tapering tracheids, the morphological trend is towards thick and stout sheathing cells around the veins or confined to vein endings as exemplified in species of *Agauria.* The next type has terminal or sub-terminal spheroidal to polygonal inflated parenchymatous cells at the vein endings in the genus *Craibiodendron.* The next distinct type is the splayed appearance of the vein-system with sheathing cells as illustrated in *Lyonia* species and also in a few species of *Pieris.*

Similarly, in the Gaultheria group one can observe certain deviations of veins and vein endings. Firstly, starting from the simple tracheids, the deviation is towards formation of sheathing cells in aggregates along the veins and also at the vein endings as seen in *Zenobia* (Lems 1964). Secondly, the straight or curved bulbous vein endings. This is a deviation over the simple conventional tracheids. Usually the bulbous ends prossess sheathing cells in multiples. This is illustrated in species of the genus *Leucothoe.* The only exception is *Leucothoe bracamorensis* in the presence of oval to spheroidal inflated parenchymatous cells at the vein endings. This is comparable and quite similar to the situation found in all the species of *Craibiodendron.* Lastly, the occurrence of strands of sclerenchymatous cells deviating from the main path and traversing into the adjacent mesophyll tissue constituting idiofibrosclereids are observed in many species of *Gaultheria, Diplycosia* and *Pernettya.*

Judd (1981) has found several anatomical characters to be taxonomically useful in *Lyonia* at both the specific and sectional levels. Similarly the present work has demonstrated a few characteristic features of diagnostic interest in veinlet endings in the tribe *Andromedeae.* It is probable, however, that further investigation should reveal more. Without information about many more species from this tribe, it is difficult critically comment on the relationship between the members of the Andromedeae from an anatomical point of veiw except that certain "groupings of species" can be seen with common characteristic features of morphogenic and probably of diagnostic interest.

Acknowledgements

The authors express their thanks to the authorities of several herbaria for the gift of leaf specimens. Thanks are also due to the UGC, New Delhi, for financial help.

References

Bocher T W 1981 Evolutionary trends in ericalean leaf structure; *Biol. Skr. K. Dan. Vidensk. Selsk.* **23** 1–64

Burtt B L 1948 Studies in the Ericales VIII; *Kew Bull.* **3** 493–495

Burtt B L, Hedge I C and Stevens P F 1970 A taxonomic critique of recent numerical studies in *Salvia* and the Ericales; *Notes R. Bot. Gard. Edinburgh* **30** 141–158

Drude O 1897 Ericaceae in Engl. and Prantl; *Die Naturlichen. Pflazen.* **4** 15–65

Hayes S F, Keenan J and Cowan J M 1951 A survey of the anatomy of the *Rhododendron* L. Leaf in relation to the taxonomy of the genus; *Notes R. Bot. Gard. Edinburg* **21** 1–51

Holmgren P K, Keuken W and Schofield E K 1981 *Index herbariorum 1. The herbaria of the world* 7th edn *Regnum Veg.* **106** 1–452

Hooker J D 1876 Ericaceae in *General plantarum* **2** (London: Reeve and Co.)

Judd W S 1979 Generic relationships in the Andromedeae (Ericaceae); *J. Arnold Arb.* **60** 477–503

Judd W S 1981 A monograph of *Lyonia* (Ericaceae); *J. Arnold Arb.* **62** 63–128; 120–209; 315–436

Lems K 1964 Evolutionary studies in the Ericaceae II Leaf anatomy as a phylogenetic index in the Andromedeae; *Bot. Gaz.* **125** 178–186

Niedenzu F 1890 Uber den anatomischen Bau der Laubblatter der Arbutoideae and Vaccinioideae in Beziehung zu ihrer systematischen Gruppierung Und geographischen Verbreitung; *Bot. Jahrb.* **11** 134–263

Rao T A and Bhattacharya J 1978 A review on foliar sclereids in angiosperms; *Bull. Bot. Surv. India* **20** 91–99

Rao T A and Bhupal O P 1973 Typology of sclereids; *Proc. Indian Acad. Sci.* **B77** 41–55

Rao T A and Chakraborti S 1982 An amplified description of a hitherto uncommon species *Craibiodendron mannii* W. W. Sm. (Ericaceae); *J. Bombay Nat. Hist. Soc.* **79** 223–225

Rao T A and Das S 1979a Leaf sclereids—occurrence and distribution in the angiosperms; *Bot. Not.* **132** 319–324

Rao T A and Das S 1979b Typology of tracheoids in Angiosperms *Proc. Indian Acad. Sci.* (*Plant. Sci.*) **88** 331–345

Rao T A and Naidu T R B 1981 On the epidermal fibre-like sclereids in the two sibling genera of the Poaceae; *Curr. Sci.* **50** 958–959

Smith W W 1911 Some additions to the flora of Burma; *Rec. bot. Surv. India* **4** 273–282

Stevens P F 1970 *Agauria* and *Agariata*—an example of tropical transatlantic affinity; *Notes R. Bot. Gard. Edinburgh* **30** 341–359

Stevens P F 1971 A classification of the Ericaceae; Subfamilies and tribes; *Bot. J. Linn. Soc.* **64** 1–53

Strain R W 1933 A study of vein endings in leaves; *Am. Midl. Nat.* **14** 374–73

Watson L, Williams W T and Lance G N 1967 A mixed data, numerical approach to angiosperm taxonomy; the classification of Ericales; *Proc. Linn. Soc. London* **178** 25–35

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 655-661
© Printed in India.

Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight

T ANANDA RAO, SWAPNA CHAKRABORTI and SILPI DAS*
Botany Department, Bangalore University, Bangalore 560056, India
* Botanical Survey of India, Howrah 711103, India

MS received 6 December 1984, revised 4 April 1985

Abstract. The present study has revealed that 24 out of 39 taxa of *Petrophile* possess diverse types of sclereids. Further, it is observed that similar types of sclereids are present at the various sectional levels as recognised by Bentham. Their morphological aspects and systematic applicability have been discussed.

Keywords. Foliar sclereids; typology; *Petrophile;* Proteaceae.

1. Introduction

This genus is confined to Western Australia and closely allied to *Isopogon* R. Br. ex Knight. There are 36 species chiefly differing from its nearest ally *Isopogon* in having cone scales firmly attached to the receptacle opening simultaneously or by force for the emission of the nuts (Bentham 1870).

Despite the reported occurrence of sclereids in a few species of *Petrophile* (Jonsson 1878–79; Solereder 1908; Rao and Bhattacharya 1978; Rao and Das 1979; Metcalfe and Chalk 1950) there is no detailed study of their typology for taxonomic considerations.

2. Material and methods

Herbarium specimens (table 1) were provided by the Central National Herbarium (CAL), Howrah, India; the National Herbarium of New South Wales (NSW), Royal Botanic Gardens, Sydney, Australia and the Western Australian Herbarium (Perth), South Perth, Western Australia. The clearing technique is after Rao and Naidu (1981), and the categorisation of sclereids is after Rao and Bhupal (1973).

3. Observation

Cleared laminae of 68 specimens belonging to 39 species have revealed that sclereids are present in 21 species namely *P. acicularis, P. carduacea, P. conifera, P. crispata, P. divaricata, P. diversifolia, P. linearis, P. longifolia, P. macrostachya, P. media, P. multisecta, P. rigida, P. semifurcata, P. striata, P. teretifolia* and *P. trifida.* They are of diffuse pattern and form distinct idioblasts in the laminae. However, in a few species, namely *P. biloba, P. colorata, P. heterophylla, P. propinqua* and *P. squamata* palosclereids infrequently sheathing the veins are observed.

Table 1. Species of *Petrophile* investigated (Bentham's classification, 1870) and references to sclereid types.

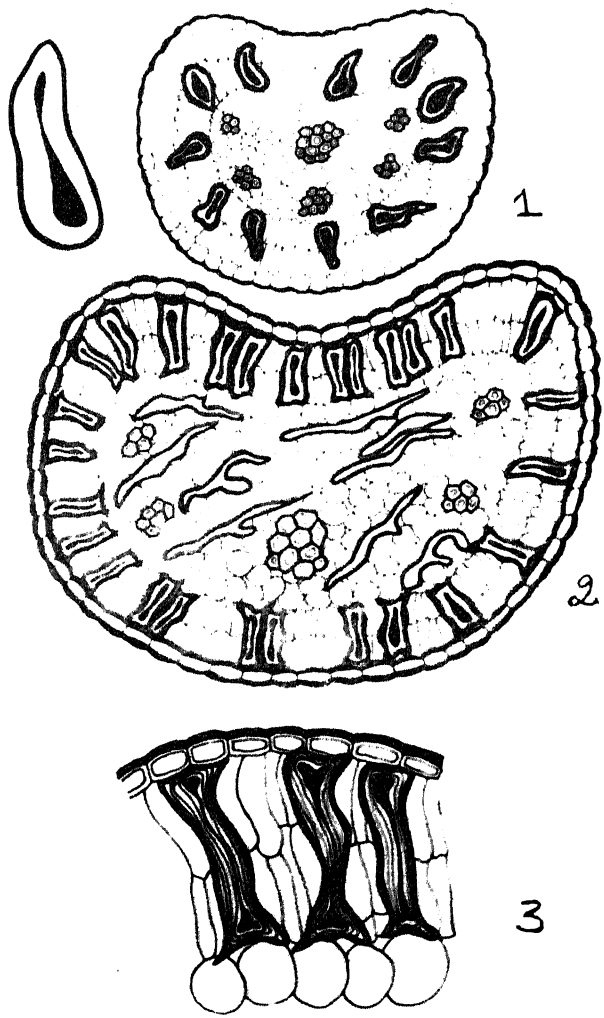
Taxa	Typology
Section 1. Arthostigma	
P. terretifolia R. Br. (J Drummond 131445, NSW)	R
P. longifolia R. Br. (D Clyae 1258151, NSW)	R
P. media R. Br. (M E Phillips 1436, NSW; Fitzgerald *s.n.*, CAL)	R, I
P. media R. Br. var *juncifolia* Lindl (E Pritzel 782, CAL; J H Maiden 75908, NSW)	R
P. acicularis R. Br. (H Sheth 75919, NSW; J Drummond 75985, NSW)	R
P. linearis R. Br. (J F Maiden 755959, NSW; B. Kaspiew 53, CAL; Max Koch 1917, CAL; E. Pritzel *s.n.* CAL)	R
P. linearis R. Br. var *anceps* R. Br. (J Drummond 75985, NSW)	R
Section 2. Xerostole	
P. heterophylla Lindl. (W. H. Archar 75987, NSW)	P
P. biloba R. Br. (J Drummond 131371, NSW; E Pritzel 503, CAL)	P
P. propinqua R. Br. (E Pritzel 592, CAL)	P
P. propinqua R. Br. var. *sericiflora* Benth. (Max Koch 1466, CAL; M E Phillips 1993, NSW)	P
P. squamata R. Br. (N Rainbow 55974, NSW)	P
P. colorata Meisn. (J Drummond 76984, NSW)	P
P. striata R. Br. (M E Phillips CBC-005512, NSW)	I
Section 3. Serrurioides	
P. divaricata R. Br. (Ashby 2683, NSW)	N
P. serruriae R. Br. (J H Maiden 75939, NSW; E Pritzel 577, CAL)	A
P. inconspicua Meisn. (J Drummond 76009, NSW)	A
P. trifida, R. Br. (J Drummond *s.n.* NSW); E M Canning 7366, NSW)	F
Section 4. Symphyolepis	
P. carduacea Meisen. (M. E. Phillips CBC-034029, NSW)	R, S
P. shuttleworthiana Meisen. (E Pritzel 729, CAL)	A
P. macrostachya R. Br. (Phillips CBC-033565, NSW; E Pritzel 181, CAL)	R, S
P. diversifolia R. Br. (Julius Scott *s.n.* NSW)	I
Section 5. Petrophile	
P. biternata Meisn. (J Drummond 113811, NSW)	A
P. plumosa Meisn. (J Drummond 113803, NSW; E Pritzel 730, CAL)	A
P. erecifolia R. Br. (Phillips CBC-037251, NSW; E Pritzel 761, CAL)	A
P. erecifolia R. Br. var. *scabriuscula* Maxim. (J H Maiden 76059, NSW; H Salsso 4195, NSW)	A
P. chrysantha Meisn. (J Drummond 13810, NSW)	A
P. pedunculata R. Br. (A A Hamilton 76149, NSW; R T Baker *s.n.* CAL)	A
P. pulchella R. Br. (L A S Johnson 76181, NSW)	A
P. sessilis Sieb. ex Sehult. (J H Garfield 76259, NSW)	A
P. fastigiata R. Br. (J W Weingley CBC-036605, NSW)	A
P. seminude Lindl. (J H Nashell 76374, NSW; E Pritzel 576, CAL)	A
P. circinata Kipp. ex Meisn. (Phillips CBC-049148, NSW)	A
P. drummondii Meisn. (Anon. s.n., CAL; D Clyne 125900, NSW)	A
P. crispata R. Br. (A C Beaugle Poole 119186, NSW)	R
P. rigida R. Br. (R Collie 76386, NSW; Max Koch 2111, CAL)	Spheroconical
P. multisecta F. Muell. (R B Bogaro 76287, NSW)	S
P. conifera Meisn. (M E Phillips 131135, NSW)	I
Section 6. Hebegyne	
P. semifuracata F. Muell. (A S George 82907, NSW)	G

Absence (A), Spheroidol or ovoid forms (S), Gnarlyform (G), I-shape (I), Ramiform (R), Fusiform (F), Nailform (N), Paloform (P).

3.1 *Typology*

The most commonly encountered sclereids conform to the following major types:

3.1a *Sub-spheroidal form*: They are observed in the laminae of *P. multisecta* (Bogero 76287, NSW) and *P. rigida* (R Collie 76384 NSW, figure 1). The sclereids chiefly conform to sub-spheroidal to sphero-conical or clavate shape. In *P. multisecta* sclereids are ovoid and appear in a row along the margin. They are more or less similar, in shape, thick-walled, and the lumen is of narrow or irregular width. The secondary wall is striated and displays many pits.



Figures 1–3. TS of the leaves of **1.** *P. rigida* R. Br. (R Collie 76384, NSW) showing sub-spheroidal sclereids disposed in palisade layer (× 225). **2.** *P. acicularis* R. Br. (H Sheth 75919, NSW) showing two types of sclereids: Palosclereids in palisade and ramiform sclereids in mid-mesophyll region (× 225). **3.** *P. media* R. Br. (M J Phillips 1436, NSW) showing I-shaped sclereids in the palisade region (× 400).

3.1b *Gnarlyform*: They have a central misshapen body with many protuberances of irregular shapes and sizes. These are observed in *P. semifuracata* (A S George 82907, NSW).

3.1c *Paloform*: They are in the form of rectangular cells and infrequently observed around the veins of all the species of the section *Xerostole* (figure 4) except *P. striata* (Phillips CBC-005512, NSW).

3.1d *I-shaped*: They are observed in the laminae of *P. conifera* (Phillips 131135, NSW), *P. divaricata* (Ashby 2683, NSW), *P. diversifolia* (Julius Scott *s.n.*, NSW), *P. media* (Phillips 1436 NSW, figure 3) and *P. striata* (Phillips CBC-005512, NSW). These sclereids are columnar with thick sclerosed walls. They have short spicules at the polar ends and are disposed parallel to one other. Their secondary cell-walls are striated and have many pits. The lumen is narrow or occluded at certain points. Under this category the sclereids of *P. divaricata* are very distinct in resembling round-headed nails (figure 9). They are closely disposed in the lamina, and often appear in rows.

3.1e *Ramiform*: This type is present in the laminae of *P. acicularis* (Sheth 75919, CAL), *P. carduacea* (Phillips 34029, NSW), *P. crispata* (Poole 119186, NSW), *P. linearis* (Maiden 755959, NSW), *P. linearis* var. *anceps* (Drummond 75985, NSW), *P. longifolia* (Clyae 1258151, NSW), *P. macrostachya* (Pritzel 181, CAL). *P. media* (Phillips 1436, NSW), *P. media* var. *juncifolia* (Maiden 75908, NSW), and *P. terretifolia* (J. Drummond 131445, NSW). These sclereids are columnar, not much-elongated but often show spicules at the ends. Most of them are arranged parallel to the leaf-surface. They form distinct rows, and sometimes occupy the sub-marginal areas of the laminae. In *P. carduacea* sclereids are oriented at right angles to the surface. In *P. linearis* var. *anceps* sclereids show distinct patterns of disposition: marginal and central. Marginal sclereids are parallel to the surface whereas central sclereids are at right angles to the surface. This feature is of diagnostic interest. In *P. acicularis* sclereids of two types are encountered: Paloform at palisade and ramiform in the central spongy portions of the cylindrical lamina (figure 2).

3.1f *Fusiform*: These are represented only in the lamina of *P. trifida* (J. Drummond *s.n.* NSW). The cell form has a bulging body with fusoid ends. They are relatively small in size with thick cell walls, and lumina of irregular width.

4. Morphological features

The species and their sclereidal forms are recorded in table 1. On the basis of total absence or presence of sclereids within the sections one can differentiate the existence of two groups of species. Of the 39 species examined under this genus, 15 have no sclereids, while the other 24 possess diverse types of sclereids. Every section recognised by Bentham (1870) shows a few species with or without sclereids except the sections *Arthostigma* and *Hebegyne*, where all the species have sclereids in their leaves. These features are neither to be considered as events of phyletic importance nor as indicators of close relationship but confirm the distinctness of the groups of species. Groups based on presence or absence of sclereids or their types alone may prove to be of little

relation to the current systematic classification. More natural groups are formed when sclereids are considered together with morphological and other histological characters.

5. Systematic applicability

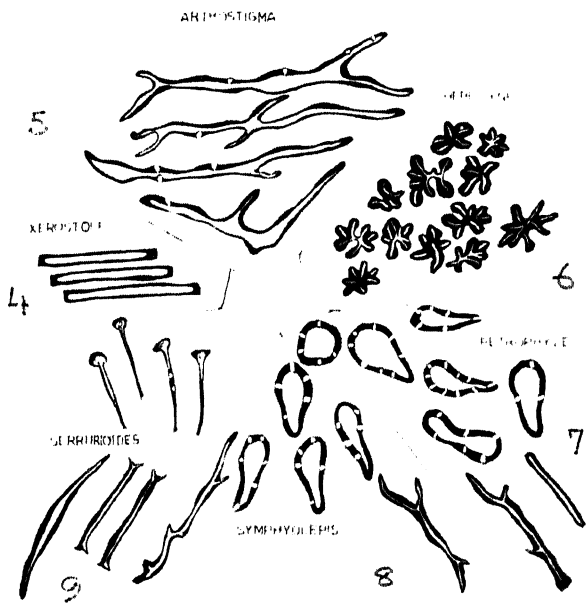
According to Bentham (1870) *Petrophile* consists of 35 species confined chiefly to Western Australia. The genus is divided into 6 sections by Bentham (1870): *Arthostigma*, *Xerostole*, *Serrurioides*, *Symphyolepis*, *Petrophile*, *Hebegyne*.

The present survey has revealed that foliar sclereids are not of generic significance but may be of diagnostic value at species levels. They provide very useful points that may be of some help in the future revision of this genus. Some of the findings of the present work are now discussed.

All taxa of the section *Arthostigma*, namely *P. teretifolia*, *P. longifolia*, *P. media*, *P. media* var. *juncifolia*, *P. acicularis*, *P. linearis* and *P. linearis* var. *anceps* exhibit ramiform sclereids. Thus, their common presence confirms this group as a natural one (figure 5).

All taxa of *Xerostole*, namely *P. heterophylla*, *P. biloba*, *P. propinqua*, *P. propinqua* var. *sericiflora*, *P. squamata* and *P. colorata* have palosclereids. The presence of distinct I-shaped sclereids in *P. striata* is undoubtedly a diagnostic character for this taxon within this section (figure 4).

Among the 16 taxa assembled under the section *Petrophile* it is possible to distinguish two sub-groups based on the presence or absence of foliar sclereids.



Figures 4–9. Typology of sclereids in different sections of *Petrophile*: Semi-diagrammatic sketches. (× 400). **4.** *Xerostole – paloform;* **5.** *Arthostigma* – ramiform; **6.** Habegyne — gnarlyform; **7.** *Petrophile* and **8.** *Symphyolepis* — spheroform to ramiform; **9.** *Serrurioides*— nailform, ramiform, fusiform.

Sclereids are absent in 12 taxa, namely *P. biternata*, *P. chrysantha*, *P. circinata*, *P. drummondii*, *P. erecifolia*, *P. erecifolia* var. *scabriuscula*, *P. fastigiata*, *P. pendunculata*, *P. plumosa*, *P. pulchella*, *P. seminuda* and *P. sessilis*. In the remaining 4 species, namely *P. conifera*, *P. crispata*, *P. multisecta* and *P. rigida* the I-shaped sclereids, ramiform sclereids, the thick ovoid or spheroidal sclereids and the sphero-conical sclereids, respectively, are of diagnostic value (figure 7).

Of the 4 species in the section *Serrurioides*, the nail-shaped sclereids of *P. divaricata* and the fusiform sclereids of *P. trifida* form distinct diagnostic cell-shapes which distinguish them from one other, and also from the two other taxa of this section which lack sclereids (figure 9).

In section *Symphyolepis* there is no difference between *P. carduacea* and *P. macrostachya* in their ramiform and spheroidal sclereids. *P. diversifolia* possesses distinct I-shaped sclereids whereas *P. shuttleworthiana* is devoid of sclereids (figure 8).

P. semifuracata is placed under a monotypic section, *Hebegyne* because of the formation of the fusiform ending of the style. Furthermore, the gnarlyform sclereids found in this species in all probability supports a distinct place for this species under this section (figure 6).

6. Taxonomic considerations

(In *P. acicularis* R. Br. the bract and style are similar to those of *P. media* R. Br. but the longitudinal ribs of the cone scales are always more prominent and the leaves more slender (Bentham 1870). Likewise the ramiform sclereids of *P. acicularis* are similar to those of *P. media* but the branching system of the sclereids in both of them are different and appears to be of diagnostic value in distinguishing them from one other.

In *P. circinata* the cone scales appear to be deciduous and the involucre large and persistent as in *Isopogon latifolus* R. Br. The perianth, style and nut are characteristic of those of *Petrophile*. The lamina of *P. circinata* is devoid of sclereids whereas in *Isopogon latifolius* there are polymorphic sclereids showing extended arms of unequal size (Rao and Das 1981).

P. erecifolia R. Br. is considered a distinct species despite its resemblence to *P. inconspicua* Meisn. or to *P. chrysantha* Meisn. The laminae of these species are devoid of sclereids and this feature is significant in the problem of their synonymy.

Regarding the species-specific status of *P. media* R. Br. and its variety *juncifolia* Lindl. the sclereidal features are more or less similar with differences only in the thickness of the walls. So they provide no additional evidence supporting their recognition as distinct taxa.

The absence of sclereid in *P. propinqua* R. Br. and its variety, *sericiflora* Benth. is a significant endomorphic feature, supporting their close relationship.

In the present study it has been found that the lamina of *P. inconspicua* is devoid of sclereids. Regarding this species Bentham (1870) is of the opinion "that it has so much of the character of *Isopogon*, that I should at once have transferred it to that genus were it not for the uncertainty which prevails about the shape and indumentum of the nut, besides that the style is much more that of the section *Serruioides* of *Petrophile* than of *Isopogon adenanthoides* Mesin. which is the nearest to the present species in *Isopogon*". *P. inconspicua* Meisn. is a very distinct species in lacking foliar sclereids whereas *Isopogon adenanthoides* Meisn. possesses gnarlyform sclereids in the laminae (Rao and

Das 1981). This point is significant in distinguishing the two species from each other despite their close resemblence.

The presence of ramiform sclereids in *P. linearis* R. Br. and its var. *anceps* R. Br. indicates their close alliance.

Bentham regards *P. crispata* R. Br. very closely allied to *P. drummondii* Meisn. and *P. rigida* R. Br. and perhaps a variety of the latter. The absence of foliar sclereids in *P. drummondii* and their presence in *P. crispata* and *P. rigida* deserve special attention in deciding their taxonomic status in future revision.

Bentham is of the opinion "that *P. conifera* Meisn. is very nearly allied to *P. rigida* R. Br., but they differ from each other with tomentose branches and longer cones". Their differentiation can also be observed in sclereid topology. In *P. conifera* Meisn. sclereids are I-shaped whereas in *P. rigida* R. Br. they are sub-spherical to conical base forms.

Acknowledgements

The authors thank the authorities of several herbaria for the gift of leaf specimens and the UGC New Delhi, for financial help.

References

Bentham G 1870 *Flora Australiensis* (London: Lovell Reeve and Co.)

Jonsson B 1878–79 Bidrag till Kannedomen om bladets anatomiska byggnad hos Proteaceerna; *Acta Univ. Lund* **15** 1–54

Metcalfe C R and Chalk L 1950 *Anatomy of the dicotyledons* (Oxford: Clarendon press) Vols 1 and 2

Rao T A and Bhattacharya J 1978 A review on foliar sclereids in angiosperms; *Bull. Bot. surv. India* **20** 91–99

Rao T A and Bhupal O P 1973 Typology of sclereids; *Proc. Indian Acad. Sci.* **B77** 41–55

Rao T A and Das S 1979 Leaf sclereids —occurrence and distribution in the angiosperms.; *Bot. Not.* **132** 319–324

Rao T A and Das S 1981 Typology and taxonomic value of foliar sclereids in the Proteaceae 1. *Isopogon* R. Br. *Proc. Indian Acad. Sci. (Plant Sci.)* **90** 31–42

Rao T A and Naidu T R B 1981 On the epidermal fibre-like sclereids in the two sibling genera of the Poaceae; *Curr. Sci.* **50** 958–959

Solereder H 1908 *Systematic anatomy of the dicotyledons* (Oxford: Clarendon Press)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 663-670
© Printed in India.

Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations

KARAN SINGH* and Y S MURTY

Department of Botany, Meerut University, Meerut 250 005, India

*Present Address: Department of Plant Physiology, Sukhadia University, Agricultural Research Station, Sriganganagar 335 001, India

MS received 2 March 1984; revised 24 September 1984

Abstract. The abscission responses of petiolar explants of NP 46-A and Pusa jwala cultivars of chilli (*Capsicum annuum* L) to proximal and distal applications of growth substances were investigated. Ethephon, abscisic acid and ascorbic acid proved to be accelerants of abscission irrespective of age of explants and site of application. The synthetic auxin (naphthalene acetic acid) retarded abscission in younger explants regardless of the site of application but in the older explants proximal application of the auxin promoted abscission while distal application retarded the process. Gibberellic acid showed effects opposite to those caused by the auxin. The proximal application of gibberellic acid retarded abscission irrespective of age of explants while distal application effectively accelerated abscission. The cytokinin (benzyl adenine) and morphactin delayed abscission of petiolar explants on distal as well as proximal applications. The abscission responses to combinations of growth substances were highly variable depending on the age of explants, site of applications and varietal source of experimental material. The morphactin and benzyl adenine exhibited additive interaction in retarding the abscission up to such extent that ethephon and abscisic acid could only partially counteract their effects in combination of two growth substances. The auxin and gibberellin showed irregular and mixed interactions with other bioregulants studied.

Keywords. Petiolar explant abscission; *Capsicum*; growth substances.

1. Introduction

Since the introduction of the method involving the use of petiolar explants (Addicott *et al* 1949) for the study of foliar abscission, various workers have used the technique for studying various aspects of abscission and its hormonal regulation (Biggs and Leopold 1957; Vendrig 1959; Jacobs 1968; Addicott 1970) under controlled conditions. Most of our present day knowledge about the role of plant growth substances in the control of abscission of plant parts is based on explant studies (Addicott 1981). However, contradictory results have been reported on the role of exogenous and endogenous bioregulants in abscission by various workers who used different plants but the same method. This is especially true for auxin (Addicott 1970), gibberellin (Chatterjee and Leopold 1964; Wittwer and Bukovac 1968) and cytokinin (Murty and Prakash 1975). Similar controversy exists regarding morphactin, ascorbic acid and some other plant growth regulators in relation to abscission. This situation prompted Jacobs (1979) to advocate more experimentations for the study of interactions of hormones, rather than the consideration of individual hormones. Such studies are quite uncommon under semi-controlled experimental conditions. Present experiments were, therefore, undertaken to make a quantitative appraisal of the abscission responses of chillies to applied growth substances and their combinations and also to assess the evidences of interactions of growth substances in the process.

2. Materials and methods

Seedlings of NP 46-A and Pusa jwala cultivars of chilli (*Capsicum annuum* L.) were raised in earthen pots and were maintained in a healthy, vigorous and one stemmed condition with uniform size of 6th fully unfolded leaf at the time of harvest. Before excision of explants, the required number of sterilized petri-dishes were half-filled with 1·5 % agar and arranged in two steel racks which were properly sterilized by 96% alcohol spray and lined with sterilised 'absorbo' blotting paper and then covered with a thin white cotton cloth. Plants at 6 leaf stage were harvested, and quickly brought to the laboratory, washed and then surface sterilized with chlorine water. Explants from nodes 1 and 5 were excised and collected in enamel trays filled with distilled water. Each explant was finally trimmed and consisted of a petiole length of 10 mm, stem length of 10 mm below the petiolar attachment and 2 mm above the petiolar attachment. Ten explants per petri dish for distal application were inserted up to 4 mm length of petiolar stump in agar. Similarly 10 explants per petri dish for proximal application were inserted in agar medium up to 4 mm of stem length (figure 1). Into each petri dish was poured 10 ml of freshly prepared solution of growth substance. The effective concentration of each of the growth substances was determined by earlier experiments (Singh 1979). The petri dishes were placed on the racks in a randomised block design pattern. Explants treated with distilled water served as 'controls'. In these experiments, no nutrient medium was used following the recommendations of Davenport *et al* (1977).

The temperature of the experimental chambers of racks varied between 24 to 27°C. Explants were subjected to natural light intensity and photoperiod prevailing in the laboratory. High relative humidity (70–80%) was maintained in all chambers so as to minimise the dessication of explants. However, few explants (marked by†† or ††† in table 1) turned brown inspite of all care. In some treatments few explants exhibited abnormally long pre-abscission periods (marked by ** or *** in the table). Pre-abscission periods of such explants were excluded from averaging so as to minimize the experimental error. Abscission of each explant was tested daily at 0800 hr with an abscission tester by gently touching the middle part of the petiole/stem stump. Laboratory experiments were conducted twice in March 1978 and then repeated in the same month of 1979. As results obtained from all the repetitions were statistically identical, data represented in figure 2 and table 1 are those obtained from finally conducted experiments (March 1979). Data were analysed by suitable statistical methods (Chandel 1978).

3. Results

A critical analysis of the quantitative characteristics of the responses of explants of both the cultivars to externally applied growth substances individually (figure 2) and in combinations (table 1) has revealed the following facts.

3.1 *Responses to individual growth substances*

The synthetic auxin (NAA-100 mg/l) accelerated abscission of petiolar explant No. 1 on proximal application. The distal application stimulated a substantial retardation of abscission in this older explant. However, the abscission of younger petiolar explant

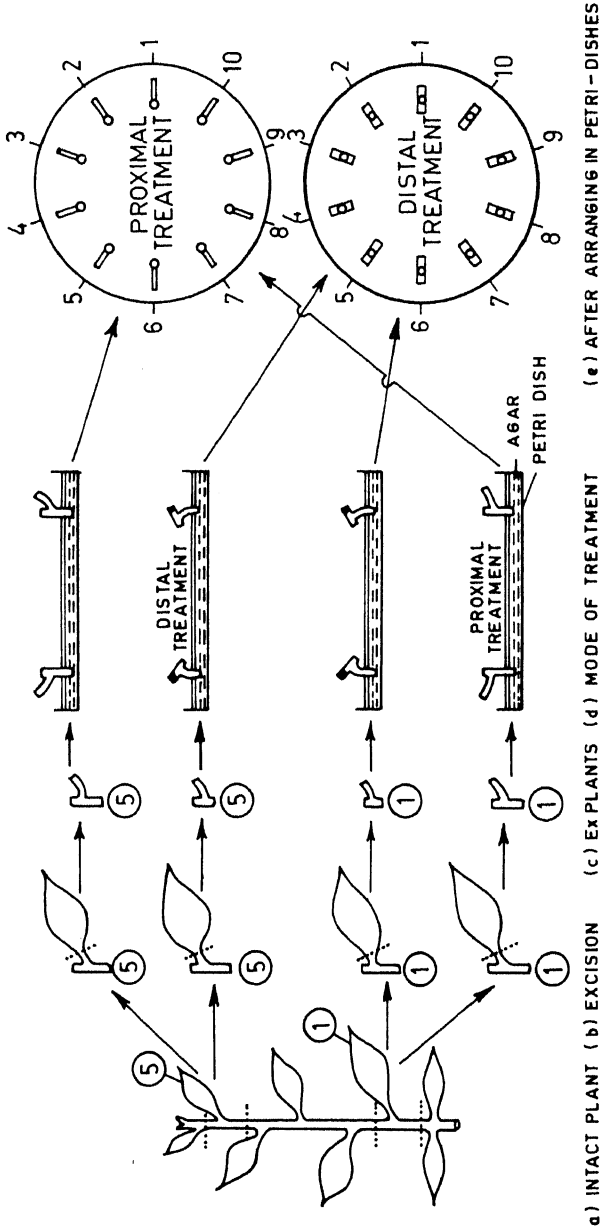


Figure 1. Diagrammatic outline of the method employed for excision, trimming and arranging petiolar explants for treatments and observations on abscission.

(No. 5) was retarded significantly by NAA applied on either sides. Benzyl adenine (BA-20 mg/l) and morphactin (CME-20 mg/l) retarded abscission of petiolar explants irrespective of explant age and mode of application in both the varieties. CME proved to be the most effective retardant of abscission (figure 2). Gibberellic acid (GA_3-100 mg/l) accelerated abscission on distal application and retarded the process when applied proximally irrespective of the age of explants. Ethephon (ETH-200 mg/l), abscisic acid (ABA-20 mg/l) and ascorbic acid (AA-200 mg/l) accelerated the abscission of petiolar explants of both ages and varieties on distal as well as proximal applications. ETH proved to be relatively the most effective accelerant of explant abscission.

3.2 *Responses to combinations of abscission retardants*

Combination of NAA and BA retarded abscission of petiolar explants of both ages and varieties on distal as well as proximal applications. The interaction of these two growth

Table 1. Pre-abscission periods (hr) of petiolar explants No. 1 and 5 in response to treatments with combinations of growth substances in *Capsicum annuum* L.

	Var. NP 46-A				Var. Pusa jwala			
	Ex. Petiole No. 1		Ex. Petiole No. 5		Ex. Petiole No. 1		Ex. petiole No. 5	
Treatments	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
Control (hr)	50·40 ± 3·15	48·44†† ± 3·39	56·50 ± 3·19	62·20 ± 3·70	46·00 ± 2·59	46.66†† ± 2·61	61·33 ± 2·39	58·80†† ± 2·49
NAA + BA	78·00 ± 3·22	109·20 ± 3·17	111·60 ± 3·26	111·60 ± 3·26	84·00 ± 2·23	96·20 ± 3·11	121·33†† ± 0·17	106·80 ± 3·14
NAA + GA	83·33†† ± 3.59	30·00 ± 2·21	94·20 ± 3·09	19·20 ± 2·78	94·80 ± 2·67	25·20 ± 2·16	94·80 ± 2·98	13·20 ± 1·26
NAA + ETH	13·20 ± 1·26	14·40 ± 1·31	26·40 ± 2·33	18·00 ± 2·67	22·80 ± 2·27	13·20 ± 1·26	22·80 ± 3·29	36·00 ± 2·33
NAA + AA	33·60 ± 2·36	97·33** ± 2·99	36·00 ± 2·66	111·60 ± 3·26	31·20 ± 3·27	94·80 ± 2·91	30·00 ± 3·26	110·40 ± 2.46
NAA + ABA	15·60 ± 1·49	13·20 ± 1·26	24·00 ± 2·59	20·40 ± 2·19	20·40 ± 2·41	24·00 ± 2·58	23·00 ± 2·17	20·40 ± 2·46
NAA + CME	130·80 ± 2·91	132·00†† ± 2·97	120·20 ± 2·78	120·00 ± 2·79	147·40 ± 3·26	130·60** ± 2·97	135·60 ± 2·99	141·44†† ± 3.2
BA + GA	86·40 ± 3·61	97·20 ± 2·31	111·80 ± 3·41	112·80 ± 3·52	96·66** ± 3·01	96·00†† ± 2·96	118·80 ± 3·27	134·40 ± 3·08
BA + ETH	24·00 ± 2·59	13·20 ± 1·20	24·00 ± 2·69	14·40 ± 1·31	13·20 ± 1·26	14·40 ± 1·68	14·40 ± 1·68	13·20 ± 1·26
BA + ABA	84·40 ± 3·31	97·50*** ± 3·13	115·20 ± 3·33	123·60 ± 2·94	88·80 ± 3·17	96·00 ± 3·21	121·20 ± 3·03	121·40 ± 3·01
BA + CME	141·33** ± 3·17	139·77†† ± 2·99	123·60 ± 2·94	121·33†† ± 3·14	130·66†† ± 3·37	141·60 ± 3·61	137·33†† ± 3·30	120·00 ± 2·98
BA + AA	97·20 ± 3·18	98·40 ± 3·13	122·20 ± 3·18	120·00 ± 3·16	134·00 ± 3·41	122·40 ± 3·29	124·80 −3·48	135·60 ± 3·44
Control (hr)	50·40 ± 3·15	48·44 ± 3·39	56·40 ± 3·19	62·20 ± 3·70	46·00 ± 2·59	46·66†† ± 2·61	61·33 ± 2·39	58·80†† ± 2·49

Table 1 (*Continued*)

	Var. NP 46-A				Var. Pusa jwala			
	Ex. Petiole No. 1		Ex. Petiole No. 5		Ex. Petiole No. 1		Ex. petiole No. 5	
Treatments	Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
GA + ETH	12·00	15·60 ±1·93	19·20 ±2·81	12·00	13·20 ±1·26	13·20 ±1·26	25·20 ±2·27	24·00 ±2·31
GA + ABA	12·00	13·20 ±1·26	24·00 ±2·59	18·00 ±2·79	22·80 ±2·11	22·80 ±2·81	13·20 ±1·26	25·20 ±2·17
GA + CME	117·80 ±2·16	121·50†† ±2·17	109·33†† ±2·98	106·80 ±2·11	93·60 ±3·38	118·80 ±3·07	91·20 ±3·00	73·33** ±2·98
ETH + AAA	84·00 ±3·19	22·80 ±2·41	96·00 ±3·17	21·60 ±2·78	73·20 ±3·10	21·60 ±2·37	96·00 ±3·21	13·20 ±1.26
ETH + ABA	32·22†† ±1·78	15·60 ±1·17	15·60 ±1·19	13·20 ±1·26	12·00	13·20 ±1·26	25·20 ±2·71	25·20 ±2·98
ETH + CME	91·20 ±3·41	88·80 ±2·91	121·20 ±3·17	122·40 ±3·41	93·80 ±3·22	88·00†† ±3·19	118·80 ±3·21	122·40 ±3·23
ETH + AA	13·20 ±1·26	13·20 ±1·26	22·80 ±2·41	23·00 ±2·36	13·20 ±1·26	21·33 ±2·79	24·00†† ±2·81	12·00
ABA + CME	121·20 ±3·17	106·50 ±2·11	94·66†† ±3·19	97·20 ±3·20	122·40 ±3·11	110·66** ±2·79	120·00 ±3·09	134·40 ±3·39
ABA + AA	21·60 ±2·39	23·60 ±2·42	12·00	26·40 ±2·39	12·00	13·26 ±1·26	21·60 ±2·13	25·20 ±3·21
CME + AA	120·00 ±3·19	117·33** ±3·25	132·00 ±3·33	97·20 ±3·28	123·60 ±3·22	95·00 ±3·42	94·55** ±3·01	78·00 ±2·78

All figures are secondary averages of 10 (except those marked by ** or *** and †† or †††).

** – Average of 9 figures
*** – Average of 8 figures
} Abnormally long pre-abscission period.

†† – Average of 9 figures
††† – Average of 8 figures
} Browned and died during experiments

LSD for NP 46-A = 6·82 (5% level of significance)
LSD for Pusa jwala = 7·39 (5% level of significance)

substances proved to be additive in enhancing the pre-abscission period. Extensive retardation of abscission by NAA + CME and BA + CME combinations also furnished the phytogerontological evidences of additive interaction of component growth substances in mixtures (table 1).

3.3 *Responses to combinations of abscission accelerants*

GA_3 when tested with ETH and ABA accelerated abscission on distal as well as proximal applications showing additive interaction in a majority of cases. However, in combination with AA, GA_3 maintained its abscission retarding action on proximal application. The accelerating action of distally applied GA_3 was not promoted by AA in NP 46-A and Pusa jwala varieties. Combinations like ETH + ABA, ETH + AA and ABA

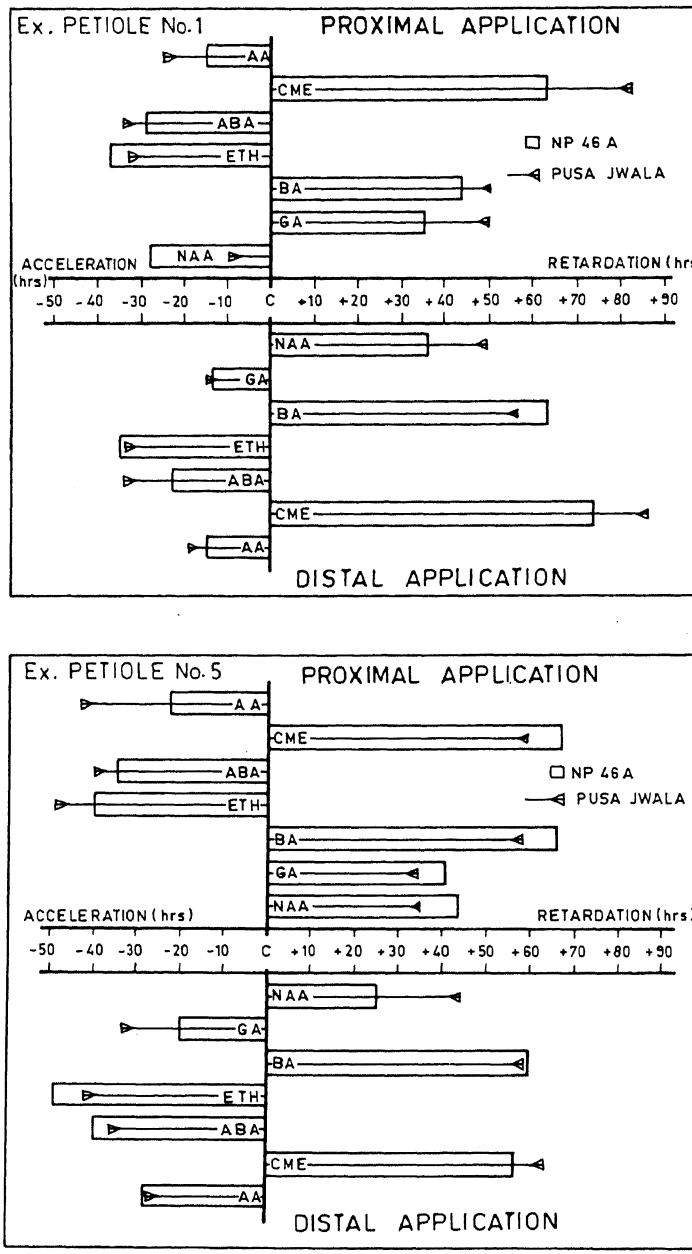


Figure 2. Percentage of changes in pre-abscission periods of *Capsicum* varieties petiolar explants in response to treatments with individual growth substances.

+ AA, though accelerated abscission significantly, none of these combinations showed additive interaction (table 1).

3.4 *Responses to combinations of abscission retardants and accelerants*

The interaction of NAA and GA_3 was much more interesting because GA_3 showed an over-riding effect on NAA action. This could be evidenced from the observations that the

retarding effect of GA_3 on proximal application and accelerating effect of GA_3 on distal application were maintained even when NAA was simultaneously applied. NAA failed to counteract significantly the strong abscission accelerating effects of ETH and ABA too. Combination of NAA with AA accelerated abscission on proximal applications while distal application retarded abscission in all cases indicating the failure of AA to reduce or modify the effect of NAA in the process of explant abscission (table 1). GA_3 could not modify the abscission retarding action of BA. BA also retarded abscission in the presence of AA and ABA, both of which could reduce its action only partially. CME strongly retarded abscission in combination with all abscission accelerants which could reduce the action of CME only insignificantly.

4. Discussion

The abscission retarding tendency of synthetic and natural auxins when applied to petiolar stumps has also been reported by several workers (Addicott 1970; Singh and Murty 1983). Louie and Addicott (1970) had concluded that factors other than auxins and auxin-gradient were involved in the regulation of abscission and Biggs and Leopold (1957) also suggested that age of explants and concentration of growth substance are important determinants of abscission response. Present studies with *Capsicum* petiolar explant system did not support the traditional auxin-gradient theory of abscission. The effects of GA_3 reported here support the findings of some earlier workers (Chatterjee and Leopold 1964; Wittwer and Bukovac 1968). Retardation of abscission by cytokinins has also been reported by Davenport *et al* (1979). Murty and Prakash (1975) have also shown a strong abscission retarding action of morphactin in cotton (*Gossypium hirsutum* L). Acceleration of abscission by ETH, ABA and AA treatments as observed in the present studies is consistent with the observations of Abeles (1973), Milborrow (1974) and Murty and Prakash (1975). The additive interaction of auxin and cytokinin in the retardation of explant abscission has also been reported by Chatterjee and Leopold (1964). The abscission responses to combinations of auxin with morphactin and of cytokinin with morphactin are in agreement with our earlier findings (Singh and Murty 1983). However, the mechanism of action and interaction of morphactin with other hormones is yet to be established (Parups 1983). The shortening of pre-abscission period of petiolar explants by gibberellin and ethylene combination was also reported by Burg (1968). The enhancement of ABA action by ETH has also been reported by Milborrow (1974). Results of interactions of GA + AA, ETH + AA and ABA + AA are in accordance with the observations of Cooper *et al* (1968) on several plant materials.

The interpretation of results of combination of NAA and GA is difficult as individually both these growth regulators have shown opposite actions in abscission on proximal and distal application. The over-riding effect of ETH in auxin and ethylene combinations was also reported by Burg (1968). The results of interactions of auxin with other abscission accelerants like ABA and AA seem to support the findings of Murty and Prakash (1975) on cotton leaf explants and of Chang and Jacobs (1973) on *Coleus* petiolar explants. The partial counteraction of the abscission retarding action of cytokinin by gibberellin was also reported by Murty and Prakash (1975) in *Catharanthus*. However, the mechanism of this interaction is still obscure (Addicott and Wiatr 1977). Partial or complete counteraction of the effect of cytokinin by abscission

accelerants like ETH, ABA and AA has also been reported by earlier workers (Abeles 1973; Milborrow 1974). Results obtained for morphactin and gibberellin interaction are similar to those reported by Murty and Prakash (1975). The responses of petiolar explants to combinations of morphactin with abscission retardants supported the findings of earlier workers (Milborrow 1974; Parups 1983). The method employed in the present investigations is simple, cheaper and can be adopted for such studies in an ordinary laboratory not having sophisticated equipments/facilities.

Acknowledgements

One of the authors (KS) is grateful to UGC for a fellowship. Thanks are due to Prof. F T Addicott of University of California for valuable suggestions and gift samples of ABA. CME was kindly supplied (as gratis sample) by CELAMERCK GmbH & Co., Germany.

References

Abeles F B 1973 *Ethylene in plant biology* (New York and London: Academic Press)

Addicott F T 1970 Plant hormones in the control abscission; *Biol. Rev.* **45** 485–524

Addicott F T 1981 *Abscission* (Berkely: University of California Press)

Addicott F T and Wiatr S M 1977 Hormonal control of abscission: Biochemical and ultrastructural aspects; in *Plant growth regulation* (ed.) P E Pilet (New York and Berlin: Springer Verlag) 249–257

Addicott F T, Lyon R S, Livingston G A and Hunter J K 1949 A method for the study of foliar abscission in vitro; *Plant Physiol.* **24** 537–539

Biggs R H and Leopold A C 1957 Factors influencing abscission; *Am. J. Bot.* **32** 626–632

Burg S P 1968 Ethylene, plant senescence and abscission; *Plant Physiol.* **43** 1500–1511

Chandel S R S 1978 *A handbook of agricultural statistics* (Kanpur: Achal Prakashan Mandir)

Chang Y P and Jacobs W P 1973 The regulation of abscission and IAA by senescence factor and abscisic acid; *Am. J. Bot.* **60** 10–13

Chatterjee S K and Leopold A C 1964 Kinetin and gibberellin action on abscission process; *Plant Physiol.* **39** 334–337

Cooper W C, Rasmussen G K, Rogers B J, Reece P C and Henry W H 1968 Control of abscission in agricultural crops and its physiological basis; *Plant Physiol.* **43** 1560–1576

Davenport T L, Morgan P W and Jordan W R 1977 Auxin transport as related to leaf abscission during water stress; *Plant Physiol.* **59** 554–557

Davenport T L, Jordan W R and Morgan P W 1979 Movement of K N and G A_3 in leaf petioles during water stress induced abscission in cotton; *Plant Physiol.* **63** 487–490

Jacobs W P 1968 Hormonal regulation of leaf abscission; *Plant Physiol.* **43** 1480–1495

Jacobs W P 1979 Plant hormones and plant development; (London: Cambridge University Press)

Louie D S Jr and Addicott F T 1970 Applied auxin gradient and abscission in explants; *Plant Physiol.* **45** 604–607

Milborrow B V 1974 The chemistry and physiology of abscisic acid; *Ann. Rev. Plant Physiol.* **25** 259–307

Murty Y S and Prakash G 1975 Effect of growth substances on the abscission of cotyledonary leaf in cotton; *J. Indian Bot. Soc.* **54** 107–109

Parups E V 1983 Physiology and biochemistry of morphactins; In *Aspects of physiology and biochemistry of plant hormones* (ed.) S S Purohit (Ludhiana and New Delhi: Kalyani Publishers) 255–290

Singh K 1979 *Abscission studies in Capsicum annuum* L., Ph.D. Thesis, Meerut University, Meerut (India)

Singh K and Murty Y S 1983 Petiolar abscission responses to hormonal treatments in *Capsicum annuum* L. varieties; *Proc. Indian Acad. Sci. (Plant Sci.)* **2** 41–49

Vendrig J C 1959 On the abscission of debladed petioles in *Coleus rhenaltianus* in relation to the effect of gravity; (Amsterdam: North Holland) 1–49

Wittwer S H and Bukovac M J 1968 The effects of gibberellins on economic crops; *Econ. Bot.* **12** 213–255

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 671-676
© Printed in India.

Foliar application of potassium on the growth and yield components of pigeonpea (*Cajanus cajan* (L.) Millsp.)

N N V S RAVINDRANATH, N V SATYANARAYANA, P PRASAD and K V MADHAVA RAO

Department of Botany, Andhra University, Waltair 530003, India

MS received 21 June 1984; revised 13 October 1984

Abstract. The effect of foliar application of potassium on the growth pattern and yield components of three cultivars of pigeonpea (*Cajanus cajan* (L.) Millsp.), were studied. Potassium increased plant height and dry matter accumulation in all the three cultivars. Days to maturity was not much effected. Potassium increased yield in all the cultivars but at different levels. Flower drop was reduced conspicuously. However among the cultivars studied, cv.PDM1 showed better response than the others.

Keywords. *Cajanus cajan*; foliar application; plant height; flower drop; yield components.

1. Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is the second-most important pulse crop of India. However, the yield potential of pigeonpea has not been fully exploited. Though it is well established that yield is intimately associated with mineral nutrition of crop plants (Kilmer *et al* 1968; Epstein 1972; Gauch 1972; Hewitt and Smith 1974), adequate information is not available on this aspect of pigeonpea (El Baradi 1978). It is generally considered that pigeonpea does not respond to potassium fertilizers (Kulkarni and Panwar 1980). Since these studies were based on soil applications, the present investigation was undertaken to study the foliar application of potassium on the growth and yield components of pigeonpea.

2. Material and methods

2.1 *Plant material*

Seeds of *Cajanus cajan* (L.) Millsp. cv.PDM1, cv.LRG30 and cv.ST1, all late maturing varieties, were obtained from APPSDC, Hyderabad. The seeds were sown 4 cm deep in 100 m^2 plots, with a spacing of 75 cm between the rows and 50 cm between the plants within the row. The potassium (K_2O) status of the soil is 310 kg/ha. In this field experiments, pigeonpea was grown as a sole crop. Certain plots were selected as controls which were sprayed with distilled water and others were considered as treatment plots, in which the plants were supplied with 10 mM KCl as a foliar spray. Foliar application of potassium during early hours of the day was carried out throughout the growth period with a 15-day interval. The crop was subjected to normal irrigation and recommended farmyard manure. For recording the data on each parameter 10 plants were collected from each plot of three replications and mean values were presented.

2.2 *Plant height*

The data on plant height measurements were collected at every 15-day interval.

2.3 *Dry matter accumulation*

For dryweight determination, the samples were kept in a hot air oven maintained at 80°C for 48 hr when constant dryweights were obtained. For the total leaf dryweight, the weight of fallen leaves were also recorded. Further an unavoidable source of error was obtained in uprooting the plants.

2.4 *Leaf area*

Leaf area was estimated by plotting the outline of leaves on a mm graph paper.

2.5 *Yield components*

The number of flowers opened and the number of pods initiated were counted every morning. After harvesting the plants the yield components like number of pods and number of seeds per plant were recorded.

3. Results and discussion

The growth pattern of three cultivars of pigeonpea is shown in table 1. Plant height increased with age in all cultivars up to 120 days and thereafter remained constant. Cultivar PDM1 always recorded higher values in height followed by cv.LRG30 and cv.ST1. Potassium treatment increased plant height in all the cultivars. It is now firmly established that potassium participates in several processes associated with growth (Kilmer *et al* 1968; Hewitt and Smith 1974). Potassium is considered to increase cell expansion resulting in growth (Dhindsa *et al* 1975; Cram 1976). The leaf area per plant increased with age up to 120 days in cv.PDM1 and cv.LRG30 whereas in cv.ST1, it continued to increase up to 135 days followed by a decline (table 1). All potassium treated cultivars showed a significant leaf area increase. The increase was 40% in cv.PDM1, 35% in cv.LRG30 and 31% in cv.ST1. Application of potassium increases cell expansion by regulating the solute potential and consequently the turgor potential (Dhindsa *et al* 1975) which may probably increase the rate of leaf area expansion and eventually the total leaf area per plant.

Dry matter accumulation of the whole plant, leaf, stem, and root increased with age in all the cultivars (figure 1). Among the cultivars PDM1 recorded higher dry matter accumulation than LRG30 and ST1. Potassium treatment increased dry matter accumulation in all the cultivars when compared to their respective control plants. Potassium supply resulted in increased shoot and root dryweights of other plants also (Barta 1982). The higher dry matter accumulation in the treatments may be due to increased photosynthesis (Moss and Peaslee 1965; Cooper *et al* 1967), followed by efficient translocation of photosynthates (Haeder and Mengel 1972) involving potassium.

The total number of flowers recorded per plant were highest in cv.PDM1. In all cultivars potassium treated plants showed low percentage of flower drop than their

Table 1. Effect of foliar application of potassium on plant height and leaf area during pigeonpea plant growth (±mean with SE).

Parameter	Variety	Age in days											
		30		60		90		120		150		180	
		CON	K^+	CON	K^+	CON	K^+	CON	K^+	CON	K^+	CON	K^+
Plant height (cm)	cv.PDM1	22·61 ±0·89	26·34 ±1·71	95·25 ±2·36	97·82 ±1·96	161·20 ±1·64	171·72 ±1·65	194·40 ±1·92	202·80 ±3·08	198·90 ±1·75	208·60 ±1·88	199·75 ±2·10	209·20 ±1·64
	cv.LRG30	20·16 ±0·78	21·70 ±1·82	91·45 ±1·41	93·25 ±3·78	162·84 ±1·72	170·52 ±1·94	191·96 ±1·55	202·68 ±2·11	196·75 1·12	203·96 ±2·07	197·12 ±3·14	205·40 ±2·87
	cv.ST1	22·44 ±1·11	24·72 ±1·21	98·53 ±1·65	103·78 ±2·05	169·40 ±1·47	176·80 ±1·50	189·56 ±2·32	194·32 ±2·20	192·70 ±1·89	199·30 ±1·53	193·40 ±1·04	200·10 ±2·56
Leaf area (cm^2/ plant)	cv.PDM1	96·81 ±9·07	142·37 ±13·77	389·69 ±9·90	576·84 ±12·25	1434·40 ±16·15	2884·21 ±11·57	2653·24 ±8.71	4418·37 ±7·21	1036·42 ±14·12	3006·95 ±7·32	776·07 ±14·21	2503·74 ±13·11
	cv.LRG30	68·70 ±6·91	105·03 ±8·42	511·68 ±12·31	939·74 ±13·00	1445·37 ±9·01	2971·25 ±10·12	2581·15 ±7·91	4007·72 ±8·05	981·42 ±12·12	2211·15 ±16·24	757·48 ±8·41	1449·69 ±9·91
	cv.ST1	76·68 ±11.12	111·79 ±14·12	585·61 ±9·21	813·01 ±7·84	1630·72 ±8·21	2653·60 ±12·21	2419·95 ±13·41	3524·20 ±9·71	1018·07 ±8·41	1431·81 ±14·56	630·66 ±10·34	1203·71 ±11·71

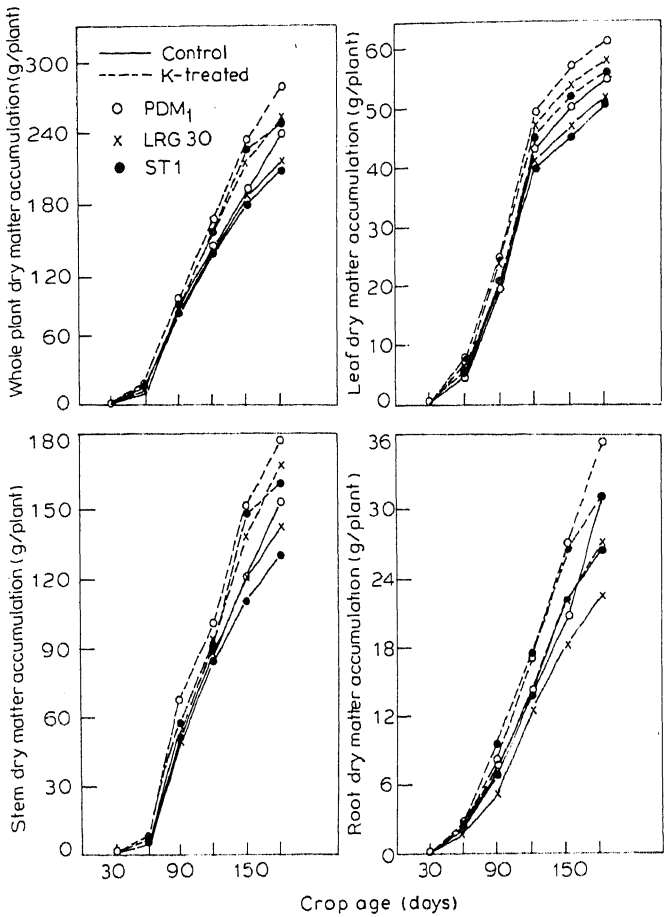


Figure 1. Effect of foliar application of potassium on dry matter accumulation of whole plant and parts of pigeonpea.

respective control plants (table 2). The flower drop recorded in control cv.PDM1, cv.LRG30 and cv.ST1 was 87·48, 84·38 and 84·42% respectively. Interestingly the flower drop reduced to 74·54% in cv.PDM1, 77·16% in cv.LRG30 and 77·48% in cv.ST1, which is always correlated with increased yield. The reduction in flower drop may be due to probable improved water relations and hormonal balance by potassium application. This response is more in cv.PDM1, followed by cv.LRG30 and cv.ST1. In all cultivars, potassium treatment resulted in increased pod number. Among potassium treated cultivars the pod number was more in cv.PDM1 followed by cv.LRG30 and cv.ST1. This is in agreement with the low percentage of flower drop in cv.PDM1. However, days to maturity and the number of seeds per pod were not altered by potassium treatment. Among potassium treated cultivars, cv. PDM1 showed higher grain yield per plant followed by cv.LRG30 and cv.ST1 (table 3). Cultivar PDM1 showed 47·73%, cv.LRG30 38·09% and cv.ST1 35·16% increase in grain yield as a result of foliar application of potassium. A similar trend of grain yield was also observed on per acre basis (table 3).

Table 2. Effect of foliar application of potassium on the flower production, flower drop and total pod number/plant in pigeonpea.

Variety	Total flower production		Flower drop %		Total pod number	
	CON	K^+	CON	K^+	CON	K^+
cv.PDM1	855	868	87·48	74·54	107·7	221·00
cv.ST1	614	631	84·42	77·48	99·83	142·00
cv.LRG30	752	762	84·38	77·16	119·22	174·33

Table 3. Effect of foliar application of potassium on yield components of pigeonpea cultivars.

Parameter	cv.PDM1		cv.LRG30		cv.ST1	
	CON	K^+	CON	K^+	CON	K^+
Leaf area (cm^2)	776·07	2503·74	757·48	1449·69	630·66	1203·71
Days to maturity	107	103	118	114	106	101
Number of pods/plant	107·7	221	119·22	174	99·83	142
Number of seed/pod	4	4	4.08	3·72	3·73	4·01
Number of seed/plant	428	884	486	647·28	370	568
Grain yield (g/plant)	31·62	60·50	31·71	51·22	29·5	45·5
Grain yield (kg/acre)	332·70	636·75	333·64	538·92	310·39	478·74

Pigeonpea showed a good response to the foliar application of potassium. Foliar nutrition may afford a remedy in situations in which the time-lag between soil application of fertilizers and plant absorption may be too long to satisfy the needs of fast growing annual crops during periods of intense growth (Noggle and Fritz 1976).

References

Barta A L 1982 Response of symbiotic N_2 fixation and assimilate partitioning to K supply in alfalfa; *Crop Sci.* **22** 89–92

Cooper R B, Blaser R E and Brown R H 1967 Potassium nutrition effects on net photosynthesis and morphology of alfalfa; *Am. Soil Sci. Soc. Proc.* **31** 231–235

Cram W J 1976 Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply; In *Encyl. Plant. Physiol.* New Series (eds) U Lutlge and M G Pitman. (Berlin, Heidelberg, New York: Springer-Verlag). Vol 2 part A 284–316

Dhindsa R S, Beasley C A and Ting I P 1975 Osmoregulation in cotton fibre; accumulation of potassium and malate during growth; *Plant Physiol.* **56** 394–398

El Baradi T A 1978 Pulses. 3. Pigeonpeas. Abstracts on tropical agriculture; in *Proc. of the Int. Workshop on Pigeonpeas* ICRISAT, Hyderabad Vol 1

Epstein E 1972 *Mineral nutrition of plants: Principles and prospectives* (New York, London: John Wiley)

Gauch H G 1972 *Inorganic plant nutrition* (Stroudsberg, Pennsylvania: Dowden, Hutchinson and Ross)

Haeder H E and Mengel K 1972 Translocation and respiration of assimilates in tomato plants as influenced by K nutrition; *Pflazenrnahr. Bodenkd* **131** 139–148

Hewitt E J and Smith T A 1974 *Plant mineral nutrition* (London: The English University's Press Ltd)

Kilmer V J, Younts S E and Brady N C 1968 *The role of potassium in agriculture*, (Madison, USA: ASA, CSSA and SSSA)

Kulkarni K R and Panwar K S 1980 Response of pigeonpea to fertilizer in India. A critical review; in *International Workshop on Pigeonpeas*, ICRISAT, Hyderabad Vol 1

Moss D N and Peaslee D E 1965 Photosynthesis of maize leaves as effected by age and nutrient status; *Am. Soil Sci. Soc. Proc.* **5** 280–281

Noggle G R and Fritz G J 1976 *Introductory plant physiology* (New Delhi: Prentice-Hall of India Pvt. Ltd)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 677-682
© Printed in India.

The pericarpic structure of *Withania somnifera* (L.) Dunal

Y S DAVE, N D PATEL and A R S MENON
Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India

MS received 1 June 1984; revised 24 January 1985

Abstract. The small berry of *Withania somnifera* is enclosed in an inflated calyx. Its pericarp is histologically differentiated into a 5–6 layered compressed collenchymatous epicarp, a multilayered zone of parenchymatous mesocarp and a 3–4 layer thick endocarp. The anomocytic stomata surrounded by 4–6 epidermal cells occur only in the basal region of the fruit. The number of cells of the mesocarp does not increase much, but its cells become enlarged, vacuolated and separated at maturity. The endocarpic lobes adhere to the placental false septa and enclose the individual seeds in separate chambers.

Keywords. *Withania somnifera*; pericarp; placentae.

1. Introduction

Withania somnifera or "Ashwagandha" as it is called in Sanskrit, is mentioned as an important drug in ancient Ayurvedic literature. Being a medicinally important plant, it has attracted the attention of various workers in the field of medicinal botany. Its seedling and floral anatomy has been worked out by Deshpande and Singh (1967). The berry of *W. somnifera* is 5–9 mm in diameter and enclosed in much enlarged inflated and a somewhat 5 angled calyx (figure 1B). Khafagy and Sabri (1970) made a pharmocognostical study of *W. somnifera* but described very little about the anatomy of the fruit. Ram and Kamini (1964) studied the embryology and fruit development of this plant, but their account does not include the structural details of the fruit.

In this paper, a detailed structural analysis of the *W. somnifera* pericarp is made in continuation of the pericarpic studies in Solanaceous fruits (Dave *et al* 1979a, b, 1980a, b, c, 1981).

2. Material and methods

Ovaries and fruits in different developmental stages were collected and fixed in FAA for 24 hr, washed in 70% alcohol to proceed by the usual methods of dehydration and infiltration. The microtomed sections (8–10 μm) stained with safranin and fast green combination (Berlyn and Miksche 1976) were made permanent by the usual procedures. The diameteres of the ovaries and developing fruit stages are shown in table 1.

3. Observations

3.1 *Ovary (from flower bud and flowers)*

The ovary is 0·5 to 1·0 mm in diameter, broad at the base and slightly narrow towards the tip (figure 1A). As seen in cross-section it is lobed and 2–3 loculed with axile

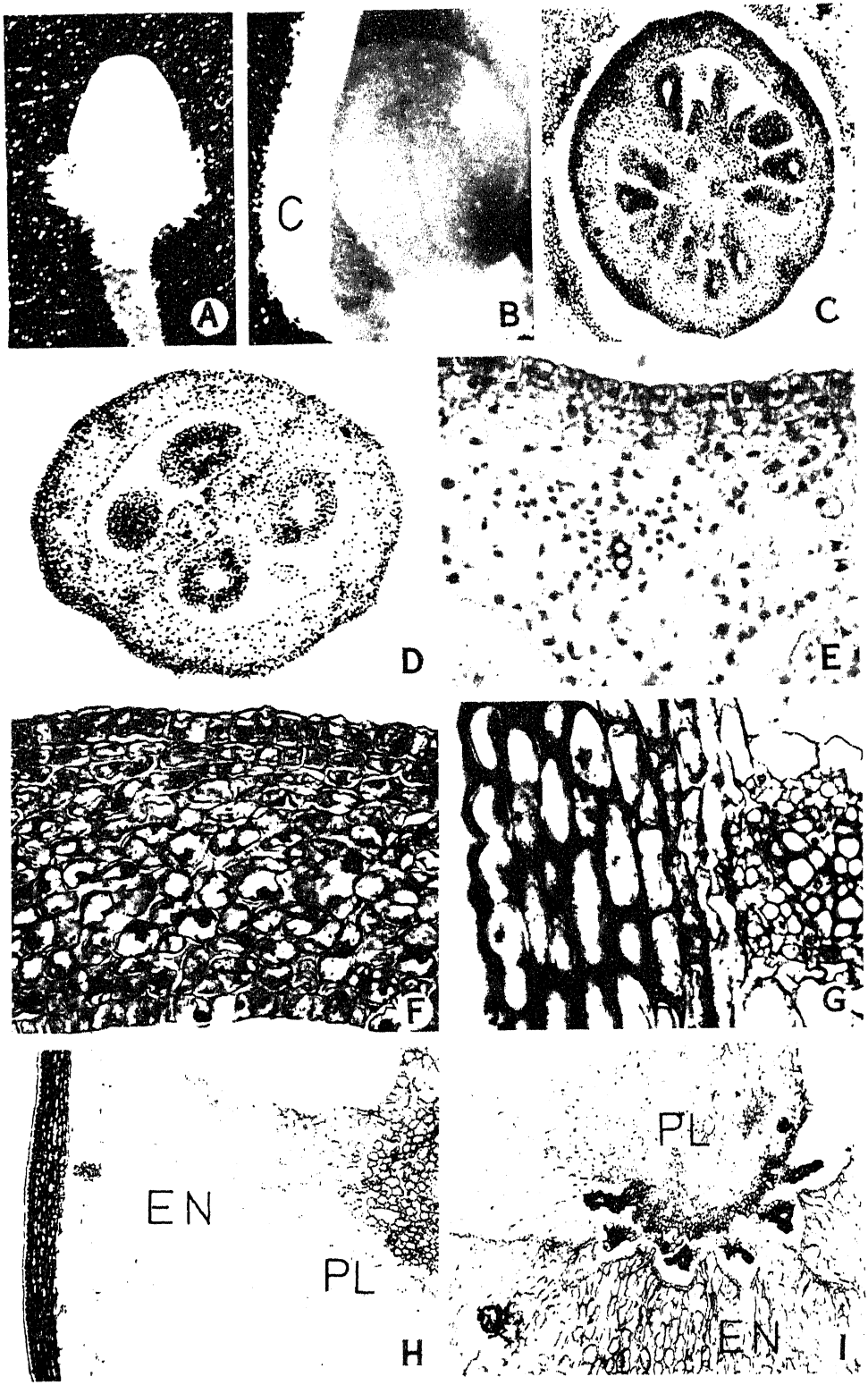

A
C
B
C
D
E
F
G
EN
PL
H
PL
EN
I

Table 1. Measurements of the ovary and fruits studied at different developmental stages.

Stage	Diameter (mm)	Remarks
1	0·5–0·7	Ovary (from flower bud)
2	0·8–1	Ovary (from flower)
3	1·5–2	Developing fruit
4	3·0–5	(green in colour)
5	5·0–9	Mature fruit (red in colour)

placentation (figure 1C). The chambers are narrow in the terminal region. The basic vascular supply is made up of four strands which branch into 12–16 in the middle of the ovary. Again above the level of the chamber the ventral and placental strands seem to join and fade out, but only two dorsals are seen extending towards the style.

3.1a *Ovary wall*: The cells in the ovary wall are almost homogeneous with large spherical nuclei and small vacuoles. The ovary wall in the bud condition is 8–9 layers thick and in the open flower stage 10–12 layers thick (figure 1E). The outer epidermis has tubular cells with thin cuticle (figure 1E). Stomata are present only in the basal region of the ovary but trichomes are absent all over.

The 2–3 layers of outer hypodermal cells appear anticlinally and periclinally divided (figure 1E). The middle zone of the ovary wall is made up of thin-walled, large vacuolated parenchyma in which the young and developing vascular bundles are embedded. There are 2–3 inner hypodermal layers with periclinally divided cells. The inner epidermis has thinly cutinized tabular cells with spherical nuclei. Stomata and trichomes are absent in the inner epidermis.

3.1b *Placentae*: There are two (figure 1C) or three axile placentae in the ovary. In the terminal region of the ovary the placentae are not seen and the two septa appear free in the centre of the unilocular chamber (figure 1D).

3.2 *Developing pericarp*

The further growth of the fruit is due to cell divisions and enlargement of the pericarpic and placental tissue.

3.2a *Epicarp*: The outer epidermis and 4–6 outer hypodermal layers constitute the epicarp of the fruit which develops from the outer epidermis and 2–3 outer

Figure 1. **A.** Ovary from open flower (× 25·6). **B.** Berry with persistant calyx (× 25·6). **C.** TS of ovary at the middle region showing axile placentation (× 108). **D.** TS of ovary at terminal region showing free septa (× 160). **E.** Cellular details of ovary wall from ventral side (× 260). **F.** Cellular details of pericarp from lateral side (× 260). **G.** Cellular details of pericarp portion with vascular bundle (× 480). **H.** Cellular details of part of pericarp septum and placenta (× 48). **I.** Portion of endocarp and placenta with aborted ovules (× 40). (C, calyx; EN, endocarp; PL, placenta)

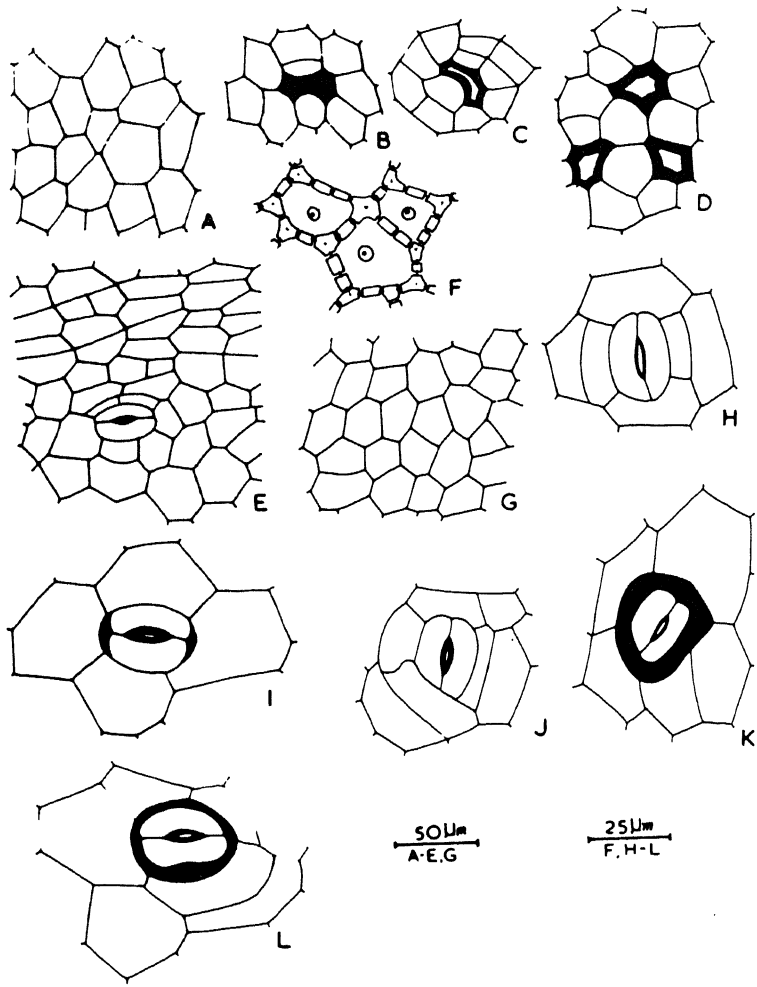


Figure 2. **A.** Outer epidermis in surface view. **B–D.** Wall thickening among the epicarpic cells. Note the formation of ventilating cleft in **C.** **E.** Outer epidermis from basal region showing the presence of stomata. **F, G.** Outer epidermal cells from the middle region of the fruit showing pitted walls. (F enlarged from G.) **H, J.** Anomocytic stomata surrounded by four epidermal cells. **I.** Thickening of polar walls of stomata. **K, L.** Thickening surrounding the stomata.

hypodermal layers of the ovary wall (figure 1G). The outer epidermis appears as polygonal parenchyma, with stomata and ventilating clefts (figures 2A, C, E, F, G, H–L). The anomocytic stomata are present only in the basal region of the fruit and are surrounded by 4–6 epidermal cells (figure 2E, H, J). Sometimes they show thickening on their polar walls or all around the guard cells (figure 2I, K, L). The epidermal cell walls in the mature stage of the fruit appear thickened and pitted, the cells enclosing cytoplasm and nuclei (figure 2F). Abnormal wall thickenings also occur in the outer epidermal cells of the developing pericarp (figure 2B, D). In transections the outer

tangential walls and the radial walls of the epidermis appear heavily cutinized (figure 1G). The 4–6 layers of outer hypodermal cells are transversely elongated or compressed and collenchymatous in nature. The whole epicarp of the mature fruit is easily peeled and separated from the inner thin walled cells of the mesocarp (figure 1G, H).

3.2b *Mesocarp*: It develops from the middle or mesodermal cells of the ovary wall. The cells do not increase much in number, but enlarge, become largely vacuolated and separated or form wide intercellular spaces towards the maturity of the epicarp (figure 1F, H, I). The mesocarpic vascular bundles are conjoint, collateral, bicollateral or concentric and embedded beneath the epicarp (figure 1G).

3.2c *Endocarp*: It develops mainly from 2–3 inner hypodermal layers and the inner epidermis of the ovary wall. During its further development, one, two or more inner hypodermal layers are produced by periclinal divisions of their walls which later undergo enlargement. They possess scanty cytoplasm and appear radially elongated and separated. The inner epidermal cells do not enlarge but elongate transversely or become compressed (figure 1I). Due to the uneven radial enlargement of the cells, the endocarpic lobes appear protruding towards the placenta in the fruit chamber (figure 1I). Some of these protruding lobes adhere with the placental septa to form individual chambers in which the seeds are enclosed.

3.3 *Placentae*

The peripheral tissue of the placentae in the early stages appear densely stained while the central cells are largely vacuolated. Later at some surface regions the placental tissue increases and enlarges to form the false placental septa which unite with the endocarpic lobes (figure 1I). The cells of the placental septa appear largely vacuolated and separated in the later stages of fruit development. The initial septa of the ovary which consists of almost isodiametric or polygonal cells with large spherical nuclei, later show elongated loosely arranged and more vacuolated cells with small elliptic or spindle shaped nuclei. The two concentric vascular bundles of the central placental axis are surrounded by largely vacuolated thin walled loosely arranged cells containing starch (figure 1H).

4. Discussion

Roth (1977) stated, "In berries which are surrounded by a protective calyx, however, the collenchyma fails to develop". But according to Klemt as quoted by Roth, the berries of Solanaceae generally develop a collenchymatous hypodermis. In contrast to Roth's statement but in accordance with Klemt's observation the pericarp of *Withania* berry consists of the epidermis and 4–6 collenchymatous hypodermal layers. Ontogenetically a single layer of the outer epidermis and 2–3 outer hypodermal layers of the ovary wall take part in its formation. The anomocytic stomata surrounded by 4–6 epidermal cells are present only in the basal region of the fruit as also found in *Physalis minima* by Patel and Dave (1976), and in *Lycium vulgare*, *Physalis alkekengi* and *Mandragora officinalis* (Roth 1977). The mesocarp of *Withania* is similar to that in *Nicandra physaloides* (Roth 1977).

The false placental septae are produced due to the limited meristematic activity and enlargement of the peripheral tissue of the placenta. The cells of the septa in mature fruit become elongated, vacuolated and separated. In some other berries like *Physalis alkekengi*, *Solanum citrullifolium*, *S. hendersoni*, and *S. nigrum* (Roth 1977), the locules are filled by proliferations of pericarp wall as well as of placentae. In *Solanum melongena* (Dave *et al* 1979b) and *S. indicum* (Dave *et al* 1980b) the multilocular condition is caused by the meristematic activity of the inner layers of the endocarp and the outgrowth from the peripheral cell layers of the placentae. No endocarpic outgrowths are found in *Solanum tuberosum* (Dave *et al* 1980c).

According to Deshpande and Singh (1967) in the lower region of the gynoecium the ovary is bilocular with axile placentation, but in the upper region the ovary becomes one-celled with apparently parietal placentation. As the locules separate from each other, the ventrals recede and the dorsals proceed to supply the style. This observation supports Puri's (1951) contention that anatomically parietal placentation is derived from axile type.

References

Berlyn G P and Miksche J P 1978 *Botanical microtechnique and cytochemistry* (Ames, Iowa: The Iowa State Univ. Press)

Dave Y S, Patel N D and Rao K S 1979a Developmental and anatomical studies in the pericarp of *Capsicums*; *Flora* **168** 263–275

Dave Y S, Patel N D and Rao K S 1979b The study of origin of pericarp layers in *Solanum melongena* L.; *Phyton* **19** 233–241

Dave Y S, Patel N D and Rao K S 1980a Morphogenesis in the fruit sculpture of *Datura innoxia*; *Phyton* **20** 159–173

Dave Y S, Patel N D and Rao K S 1980b Anatomical studies in the developing fruit of *Solanum indicum* L.; *Ceylon J. Sci. Biol. Sci.* **15** (in press)

Dave Y S, Patel N D and Rao K S 1980c Structural design of the fruit of *Solanum tuberosum* L.; *Proc. Indian Acad. Sci. (Plant Sci.)* **89** 369–374

Dave Y S, Patel N D and Rao K S 1981 Structural design of the developing fruit of *Nicotiana tabaccum* L.; *Phyton* **21** 63–71

Deshpande B P and Singh A 1967 Seedling and floral anatomy of *Withania somnifera* Dunal; *J. Indian Bot. Soc.* **46** 76–81

Khafagy S M and Sabri N N 1970 Pharmocognostical study of *Withania somnifera* Dunal I. Morphology and histology of the flower and fruit; *J. Pharm. Sci. U.A.R.* **11** 61–79

Patel N D and Dave Y S 1976 Stomata in the pericarp of *Datura innoxia* Mill., *D. metal* L. and ventilating pores of *Physalis minima* L.; *Flora* **165** 61–64

Puri V 1951 The role of floral anatomy in the solution of morphological problems; *Bot. Rev.* **17** 471–553

Ram H Y M and Kamini I 1964 Embryology and fruit development in *Withania somnifera* Dunal; *Phytomorphology* **14** 574–584

Roth I 1977 *Fruits of angiosperms* (Berlin: Gebrüder Borntraeger)

Proc. Indian Acad. Sci. (Plant Sci.), Vol. 94, Nos 4-6, June 1985, pp. 683-694
© Printed in India.

Effect of different growth substances on the foliar stomata of *Tagetes erecta* L

VIDHU, R K SHARMA* and Y S MURTY

Botany Department, Institute of Advanced Studies, Meerut University, Meerut 250 005, India

*Present address: Botany Department, SSV (PG) College, Hapur, Ghaziabad, India

MS received 13 December 1983

Abstract. The response of abaxial and adaxial stomata to various growth substances were examined in leaves of *Tagetes erecta* L. From stomatal studies it is clear that growth substances produced several variations in the morphology of stomata. The anomalies recorded include persistent stomatal initial, loss of one or both guard cells in a significant percentage of stomata, contiguous stomata, cytoplasmic connections, one and a half contiguous stomata etc. Significant changes in frequency of epidermal cells and stomata, size of epidermal cells and stomata and stomatal index were noticed in various treatments.

Keywords. *Tagetes erecta*; stomata; growth substances.

1. Introduction

The influence of various growth regulators, environmental factors, mechanical injury etc. on the structure and development of stomata has been under study for the last three decades. Inamdar (1970) investigated the action of growth regulators on the development of stomata in the cotyledons of *Abelmoschus esculentus* Moench. Cooper *et al* (1972) studied the interaction of abscisic acid and kinetin on the stomata of barley. Effect of growth regulators—IAA and TIBA, on the structure and ontogeny of stomata is observed by Inamdar *et al* (1974). Kasat (1979) studied the effect of growth regulators on the stomatal development in *Vigna sinensis*. The growth regulators are also reported to affect the frequency of stomata, stomatal index and the size of epidermal and guard cells but not the ontogeny of stomata which seems to be a genetic character as also noticed by Sharma and Dunn (1968). For the present study *Tagetes erecta* L. of the family compositae was used. Observations are presented on the action of various growth substances—indole-3-acetic acid, gibberellic acid, 2,3,5-triiodobenzoic acid and kinetin.

2. Material and methods

The leaves that emerged after spray treatment with different growth substances were collected, washed thoroughly and fixed in FAA. The following growth substances were used: indole-3-acetic acid, gibberellic acid, 2,3,5-triiodobenzoic acid in 50, 100, 200 and 500 ppm concentrations and kinetin in 10, 25, 50 and 100 ppm concentrations.

Epidermal peels taken by the direct peel method from the middle region of the terminal leaf segments were stained in 1 % aqueous solution of safranin and mounted in glycerine. Camera lucida drawings were prepared showing the exact size, shape and number of stomata on the epidermal peels. The stomatal index, diversity of stomatal

types and stomatal frequency were calculated from ten different peels of each of the surfaces in each concentration at a uniform magnification. The stomatal index is calculated as defined by Salisbury (1927, 1932) *viz.* $100S/(E+S)$, where S is the number of stomata and E the number of epidermal cells over a given surface per unit area. The length and breadth are measured at random for 25 stomata in each concentration on both surfaces. Since the epidermal cells are variously oriented, their longest and broadest distances (here considered as length and breadth) are also measured in the same way as those of the stomata. Length/breadth ratio of stomata from both the surfaces is calculated (Solereder 1908).

3. Observations

3.1 *Mature epidermis*

The epidermal cells are polygonal isodiametric or elongated with mostly sinuous (figures $1A_1$, C_3, G_4; $2D_1$, D_2; $3C_1$), rarely straight (figures $1F_{10}$; $2C_7$, D_5; $3A_1$, A_2, B_1, E_1, F_1, F_4 and F_5) or arched anticlinal walls (figures $1B_4$; $2A_4$, D_1; $3D_1$). The stomata are distributed, all over the surfaces, except over the veins without any definite pattern of orientation.

3.2 *Mature stomata*

The great majority of stomata are anomocytic in the control and other treatments (figures $1A_1$, B_4, E_2, F_{10}; $2A_4$, C_7, D_1, D_2, D_5; $3C_1$, D_1, E_1). Diacytic stomata are also observed in GA_3 100 ppm on the abaxial side besides the normal type (figure $1G_4$). Sometimes anomocytic stomata surrounded by three epidermal cells simultating an anisocytic type, are observed on the abaxial surface of IAA 100 ppm, on both adaxial and abaxial surfaces of Kn 50 ppm and on adaxial surface of Kn 100 ppm treatments (figures $1C_3$; $3A_1$, A_2, B_1). The stomatal pore may be small lenticular (figures $1A_1$, A_2) or oval as observed in TIBA 50 ppm (figure $3C_2$) or abnormally big as observed in IAA 200 ppm, GA_3 50 ppm, Kn 50 ppm and TIBA 100 ppm (figures $1D_1$, F_2; $3A_1$, D_2). The anomocytic stomatal apparatus contains a pore enclosed by two reniform guard cells surrounded by 4–6 ordinary epidermal cells (figures $1A_1$, E_2, F_{10}; $2A_4$, D_1, D_3, D_5). The development of anomocytic stomata is as follows that the meristemoid directly functions as a guard mother cell without cutting off any subsidiary cell. It enlarges, becomes rounded and divides by a straight wall to form a pair of guard cells. A lenticular pore develops in between the two guard cells. In diacytic stomata the stomatal mother cell divides by a straight wall at right angles to the subsidiary cells to form a pair of guard cells.

3.3 *Abnormalities*

In addition to normal stomata various aberrant types are induced by growth substances. They are as follows:

3.3a *Persistent stomatal initial*: The persistent stomatal initials are observed in all concentrations of gibberellic acid used (figures $1F_5$, F_8, F_9, G_6; $2A_5$, B_2), in TIBA 200 ppm (figures $3E_2$, E_4) and 500 ppm (figures $3F_2$, F_4). These initials may occur

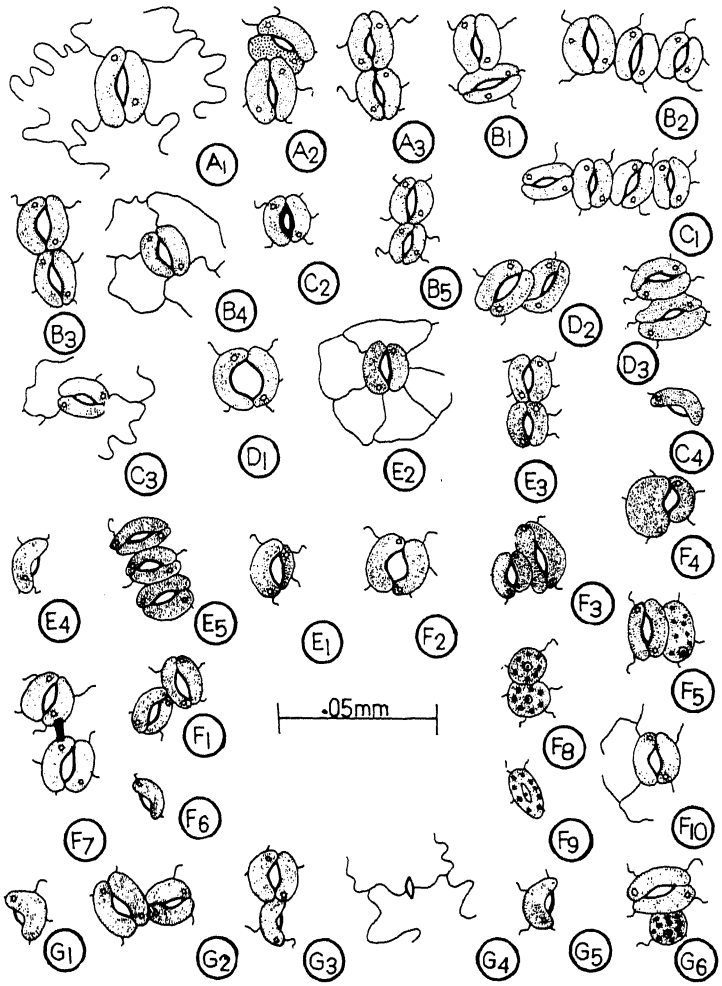


Figure 1(A–G). Effect of growth substances on stomata. A_1–A_3. Control. Portions of abaxial leaf epidermis. B_1–B_3. IAA 50ppm. Portions of adaxial leaf epidermis. B_4–B_5. IAA 50 ppm. Portions of abaxial leaf epidermis. C_1–C_2. IAA 100 ppm. Portions of adaxial leaf epidermis. C_3–C_4. IAA 100 ppm. Portions of abaxial leaf epidermis. D_1–D_2. IAA 200 ppm. Portions of adaxial leaf epidermis. D_3. IAA 200 ppm. Portions of abaxial leaf epidermis. E_1–E_3. IAA 500 ppm. Portions of adaxial leaf epidermis. E_4–E_5. IAA 500 ppm. Portions of abaxial leaf epidermis. F_1–F_6. GA_3 50 ppm. Portions of adaxial leaf epidermis. F_7–F_{10}. GA_3 50 ppm. Portions of abaxial leaf epidermis. G_1–G_3. GA_3 100 ppm. Portions of adaxial leaf epidermis. G_4–G_6. GA_3 100 ppm. Portions of abaxial leaf epidermis.

either solitary (figures $1F_9$; $2A_5$; $3E_4$, F_4) or contiguous with each other (figures $1F_8$; $2B_2$; $3E_2$) or with normal stomata (figures $1F_5$, G_6; $2C_8$; $3C_3$, E_1, F_2). The shape of the persistent stomatal initial may be spherical (figures $1F_8$, G_6; $2A_5$, B_2, C_8; $3C_3$) or ellipsoidal (figures $1F_5$, F_9; $2E_2$). Sometimes the persistent stomatal initial develops a much thickened wall (figure $3E_4$). Such initials may be caused because the meristemoid is inhibited in its further development. Such inhibited meristemoid cells fail to divide or give rise to a pair of guard cells with chloroplast and thick wall.

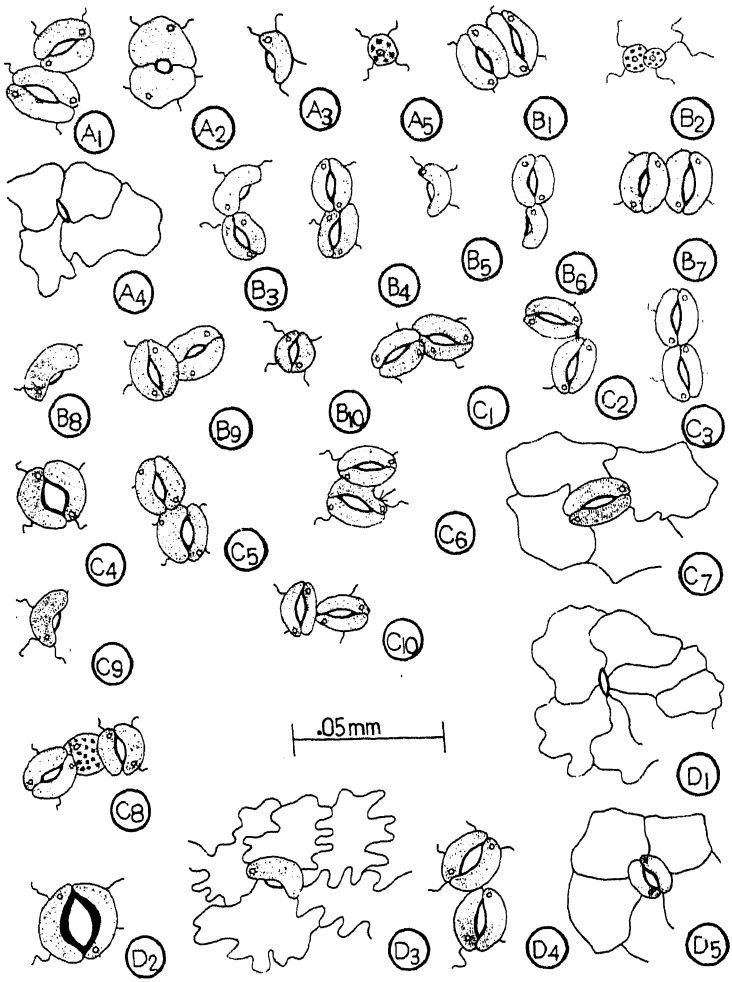


Figure 2(A–D). A_1–A_3. GA_3 200 ppm. Portions of adaxial leaf epidermis. A_4–A_5. GA_3 200 ppm. Portions of abaxial leaf epidermis. B_1–B_6. GA_3 500 ppm. Portions of adaxial leaf epidermis. B_7–B_{10}. GA_3 500 ppm. Portions of abaxial leaf epidermis. C_1–C_3. Kn 10 ppm. Portions of adaxial leaf epidermis. C_4–C_{10}. Kn 10 ppm. Portions of abaxial leaf epidermis. D_1–D_2. Kn 25 ppm. Portions of adaxial leaf epidermis. D_3–D_5. Kn 25 ppm. Portions of abaxial leaf epidermis.

3.3b *Contiguous stomata*: Variously oriented contiguous stomata were observed in control as well as in various treatments. Their frequency was more in IAA and TIBA treatments than in the control and other treatments. Two to four contiguous stomata (figures $1A_2$, B_2, C_1, E_5) were either juxtaposed (figures $1A_2$, B_1, B_2, C_1, F_1; $2A_1$, B_1, B_7; $3C_4$, F_6) superimposed (figures $1A_3$, B_3, B_5, E_3, E_5; $2B_4$, C_3, C_5, D_4; $3D_4$, E_3) or at right angles (figures $2B_9$, C_{10}) or obliquely oriented (figure $3B_4$) are also observed. Contiguous stomata may be equal (figure $1A_2$) or unequal in size (figure $1F_3$, G_2). Contiguous stomata develop after two or four stomata come in contact with each other and becomes contiguous as a result of readjustment of the epidermis during maturation.

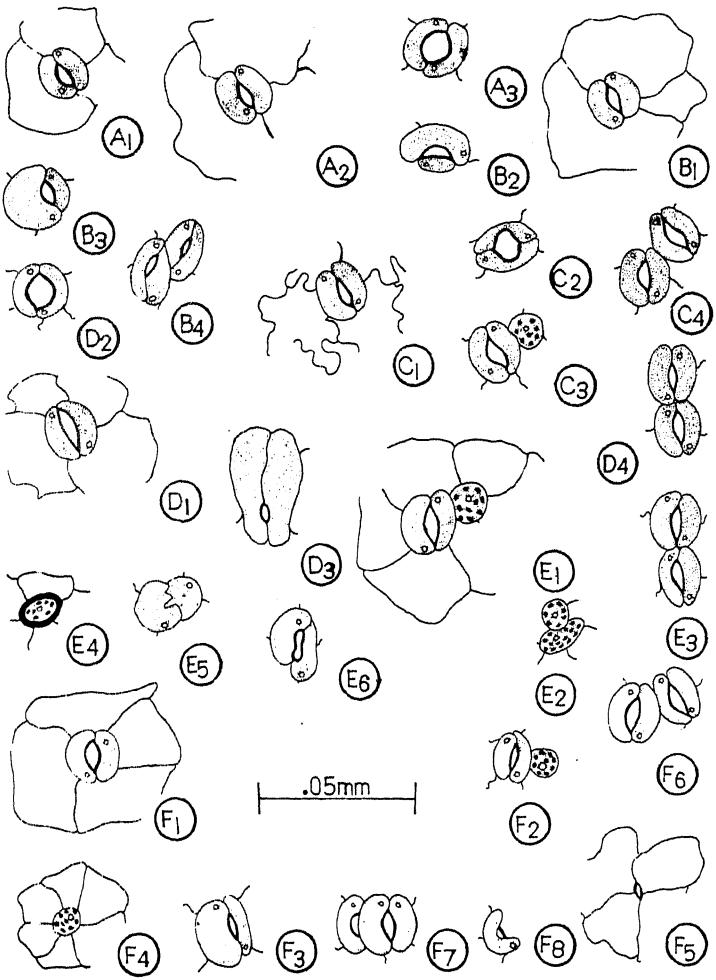


Figure 3 (A–F). A_1. Kn 50 ppm. Portions of adaxial leaf epidermis. A_2–A_3. Kn 50 ppm. Portions of abaxial leaf epidermis. B_1–B_2. Kn 100 ppm. Portions of adaxial leaf epidermis. B_3–B_4. Kn 100 ppm. Portions of abaxial leaf epidermis. C_1–C_3. TIBA 50 ppm. Portions of adaxial leaf epidermis. C_4. TIBA 50 ppm. Portions of abaxial leaf epidermis. D_1–D_2. TIBA 100 ppm. Portions of adaxial leaf epidermis. D_3–D_4. TIBA 100 ppm. Portions of abaxial leaf epidermis. E_1–E_3. TIBA 200 ppm. Portions of adaxial leaf epidermis. E_4–E_6. TIBA 200 ppm. Portions of abaxial leaf epidermis. F_1–F_3. TIBA 500 ppm. Portions of adaxial leaf epidermis. F_4–F_8. TIBA 500 ppm. Portions of abaxial leaf epidermis.

3.3c *Cytoplasmic connection*: Cytoplasmic connections connect the protoplasts of adjacent guard cells of nearby stomata. These were observed in GA_3 50 ppm (figure $1F_7$) and Kn 10 ppm (figure $2C_2$).

3.3d *One and a half contiguous stomata*: These were observed in various treatments in which a single guard cell is contiguous with a normal stomata (figures $1G_3$; $2B_3$, B_6; $3F_7$).

3.3e *Single guard cell*: Single guard cells were observed in various treatments (figures $1C_4$, E_4, F_6, G_1, G_5; $2A_3$, B_5, B_6, B_8, C_9, D_3; $3F_8$). In the development of single guard cell the meristemoid directly differentiates into a single guard cell instead of giving rise to a pair of guard cells. It enlarges, get notched on one side and becomes bean shaped with differential wall thickenings on the side of the notch.

3.3f *Degenerated guard cells*: Both the guard cells of stomata have degenerated and thus having only a pore were observed in GA_3 100 ppm (figure $1G_4$) GA_3 200 ppm (figure $2A_4$) TIBA 500 ppm (figure $3F_5$) and Kn 25 ppm (figure $2D_1$). During the process of degeneration the guard cell nucleus degenerates and disappears followed by cytoplasm and guard cell wall leaving only a pore with thickening.

3.3g *Other abnormalities*: Other abnormalities such as transversely divided guard mother cell with a small opening in the centre (figure $2A_2$), unequal guard cells (figures $1E_1$, F_4; $3B_3$, F_3), giant stomata (figure $2C_4$, D_2), stomata without pore (figure $3E_5$), obliquely oriented guard cells (figure $3E_6$), one guard cell encroached upon by an adjoining epidermal cell (figure $2C_6$), eccentrically situated pore on elongated guard cells (figure $3D_3$), one guard cell enlarged and reniform, the other small and straight (figure $3B_2$), pore side with thickened wall (figure $2D_2$) were also observed.

3.4 *Frequency of epidermal cells*

The number of epidermal cells on both adaxial and abaxial surfaces were counted in a unit area (table 1). A general pattern was observed on the abaxial surface where 50 and 100 ppm of IAA and GA_3 showed promotion in the frequency of epidermal cells while their 200 and 500 ppm showed an inhibition. However, TIBA and Kn had an inhibitory effect in 50 ppm and 10 ppm on frequency of epidermal cells while it progressively increased in 100, 200 and 500 ppm of TIBA and 25, 50 and 100 ppm of Kn. Maximum number of epidermal cells per unit area was found in Kn 100 ppm, *i.e.* 186·44 and minimum in IAA 500 ppm, *i.e.* 90·39. On the adaxial surface, frequency of epidermal cells showed a different pattern from that of abaxial surface. IAA decreased the frequency of epidermal cells as concentration increased. In an unit area 201·50 and 186·44 epidermal cells were found in GA_3 50 and 500 ppm while 109·22 and 101·69 cells were found in 100 and 200 ppm. In TIBA treated plant, the frequency of epidermal cells increased with increasing concentrations. In 10 ppm, only 94·16 epidermal cells were present in an unit area, while in 25, 50 and 100 ppm, frequency of epidermal cells increased with increasing concentration reaching 190·2 mm^{-2} in 100 ppm.

3.5 *Frequency of stomata*

It is defined as the number of stomata per unit area. On both the surfaces, it was found that all treatments increased the number of stomata per unit area except IAA 500 ppm where slight inhibition was found over control (table 1). IAA and kinetin increased the frequency of stomata but the frequency decreased with increasing concentrations on both the surfaces. GA_3 showed a different pattern with regard to the number of stomata on the abaxial and adaxial surfaces as far as concentration is concerned. On the abaxial surface it increased from 50 ppm to 500 ppm while on the adaxial surface it decreased from 50 ppm to 500 ppm. The frequency increased with increase in concentration of TIBA.

Table 1. Effect of different growth substances on epidermal characters in *T. erecta* L.

Treatment	Concentration (ppm)	Frequency of epidermal cells (mm^{-2})		Frequency of stomata (mm^{-2})		Stomatal index	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Control	0	111·11 ±2·07	116·76 ±3·07	28·25 ±3·23	15·07 ±0·75	23·44	11·43
IAA	50	122·41 ±3·08	112·99 ±4·05	41·43 ±2·32	24·48 ±1·07	25·29	17·81
	100	112·99* ±1·09	111·11 ±5·05	35·78 ±1·04	22·60 ±0·99	24·05	16·90
	200	109·22* ±4·07	97·92 ±0·79	30·13 ±1·00	20·72 ±0·98	21·62	17·46
	500	90·36 ±2·69	90·39 ±1·89	26·37 ±0·97	13·18 ±0·34	22·58	12·73
GA_3	50	120·52 ±7·08	201·50 ±8·20	33·90 ±1·08	33·90 ±0·84	21·95	14·40
	100	111·11** ±2·08	109·22 ±0·99	38·25 ±1·07	30·13 ±0·73	25·31	21·62
	200	101·69 ±1·07	101·69 ±2·33	47·08 ±2·04	22·60 ±0·72	31·65	18·18
	500	97·92 ±6·04	186·44 ±3·86	54·61 ±1·06	22·60 ±0·81	35·80	10·81
TIBA	50	109·22 ±2·02	137·47 ±4·49	37·66 ±2·07	24·48 ±0·93	25·64	15·12
	100	111·11** ±3·04	150·65 ±12·23	48·96 ±1·09	20·72 ±0·46	30·59	12·09
	200	112·99* ±2·08	158·19 ±12·07	45·20 ±1·21	41·43 ±1·08	32·43	20·75
	500	141·24 ±3·07	167·60 ±15·05	60·26 ±3·27	43·32 ±1·07	29·91	20·54
Kn	10	109·22* ±4·89	94·16 ±1·08	52·73 ±2·09	54·61 ±2·03	32·56	35·90
	25	122·41 ±9·08	148·77 ±4·37	50·85 ±1·89	45·20 ±1·08	29·35	25·47
	50	139·35 ±2·34	167·60 ±10·85	43·31 ±1·25	37·66 ±0·94	23·71	20·54
	100	186·44 ±10·28	190·20 ±12·37	39·55 ±1·30	26·37 ±0·25	17·50	17·21

* Insignificant at 1%.
** Insignificant at 1 and 5%.

3.6 *Stomatal index*

It is more on the abaxial surface in all treatments than on the adaxial surface except in Kn 10 ppm where stomatal index was more on the adaxial surface. Maximum stomatal index was found in GA_3 500 ppm, *i.e.* 35·80 and minimum in Kn 100 ppm, *i.e.* 17·50 on the abaxial surface while on the adaxial surface maximum stomatal index was found in Kn 10 ppm *i.e.* 35·90 and minimum in GA_3 500 ppm, *i.e.* 10·81. (table 1).

3.7 *Epidermal cell size*

From the data it is clear that epidermal cell size was almost equal in control on both the adaxial and abaxial surfaces. On abaxial surfaces IAA 50 ppm decreased both length and breadth of epidermal cells, IAA 100 ppm decreased the length but breadth was not affected. In IAA 200 and 500 ppm epidermal cell size increased both length and breadth wise while on adaxial surfaces there was increase in size of epidermal cells as concentration increased. On abaxial and adaxial surfaces in GA_3 treated plants length of the epidermal cells gradually decreased with increasing concentration and ultimately became equal to that of control in 500 ppm. Breadth of epidermal cell was also affected by GA_3 treatment. In 50 ppm it was less while in 100 and 200 ppm it was more than that of control. In TIBA treatment, on both abaxial and adaxial surfaces both length and breadth decreased as concentration increased. In kinetin treatment all concentrations in the increasing order showed inhibitory effect on length and breadth on both the surfaces except Kn 10 ppm where a promotion in length and breadth of epidermal cells on both the surfaces was observed (table 2).

3.8 *Stomatal size*

Control plants had stomata which were 26·64 μ in length and 19·98 μ in breadth on the abaxial side while on the adaxial side stomata had an average length of 26·64 μ and breadth of 16·65 μ. On the abaxial surface, the length of stomata increased in 50 and 100 ppm IAA while it decreased in 200 and 500 ppm IAA. Breadth of the stomata remained same as in control except of 50 ppm. In general, both length and breadth of stomata on the adaxial surface showed reduction as concentration of IAA increased except in 50 ppm where there was no difference in comparison to control.

As far as stomatal size of GA_3 treated plants is concerned, on the abaxial side different concentrations except 500 ppm showed same length of stomata but width was greatly affected. On the adaxial surface length of stomata was reduced in all concentrations while breadth decreased in 50 and 100 ppm but was equal to control in 200 and 500 ppm. In TIBA, lower concentrations did not affect the length of stomata on the abaxial surface as it remained equal to that of control but the breadth showed reduction. On the adaxial surface as concentration increased, decrease in length was observed. In TIBA 50 ppm, the length and breadth were equal to that of control. In kinetin treatment, on both adaxial and abaxial surfaces length and breadth increased linearly as concentration increased except in Kn 10 ppm on the adaxial surface where breadth was reduced to nearly half of that of the control (table 2).

3.9 *Stomatal length/breadth ratio*

It was calculated for both the surfaces separately. In control plants, on the abaxial surface, the ratio was 1·33 and on the adaxial surface it was 1·6. The ratio was 1·63, 1·66, 1·16 and 1·33 on the abaxial surface and 1·6, 1·5, 1·5 and 1·75 on the adaxial surface in 50, 100, 200 and 500 ppm IAA respectively. In GA_3 treated leaf the ratio was 1·4, 1·75, 1·16 and 1·1 on the abaxial surface and 1·25, 1·5, 1·4 and 1·33 respectively on the adaxial surface in 50, 100, 200 and 500 ppm. TIBA and Kn concentrations increased the ratio on both the surfaces (table 2).

Table 2. Effect of different growth substances on epidermal characters in *T. erecta* L.

Treatment	Concentration (ppm)	Epidermal cell size				Stomatal size				Stomatal length/breadth ratio	
		Abaxial		Adaxial		Abaxial		Adaxial			
		Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Abaxial	Adaxial
Control	0	56·61 ±2·39	26·64 ±2·04	56·61 ±3·02	24·98 ±1·04	26·64 ±1·07	19·98 ±0·23	26·64 ±1·01	16·65 ±1·04	1·33	1·60
IAA	50	48·29 ±3·05	23·31 ±1·03	56·61** ±2·07	26·64 ±1·06	29·97 ±2·08	18·32 ±0·46	26·64** ±1·01	16·65** ±1·04	1·63	1·60
	100	53·28 ±2·07	26·64** ±1·04	59·95 ±2·09	24·98** ±1·04	33·30 ±2·30	19·98** ±0·54	26·31** ±1·02	16·65** ±1·04	1·66	1·50
	200	59·94 ±4·09	28·31 ±1·21	61·61 ±3·06	26·64 ±1·06	23·31 ±0·99	19·98** ±0·96	23·31 ±0·99	14·99 ±0·88	1·16	1·50
	500	63·27 ±5·95	28·31 ±1·22	63·27 ±4·04	28·31 ±0·99	26·64** ±1·07	19·96** ±0·96	23·31 ±2·08	13·32 ±0·44	1·33	1·75
GA_3	50	66·60 ±5·23	29·97 ±1·32	46·62 ±3·05	22·48 ±0·42	23·31 ±1·06	16·65 ±0·32	24·98 ±1·07	19·98 ±0·67	1·40	1·25
	100	64·94 ±4·98	28·31 ±0·97	56·61** ±3·07	26·64 ±0·94	23·31 ±1·08	13·32 ±0·21	29·97 ±2·39	19·98 ±0·67	1·75	1·50
	200	59·94 ±3·29	23·31 ±0·45	56·61** ±3·08	26·64 ±0·93	23·31 ±0·75	19·98** ±1·08	23·31 ±1·04	16·65** ±1·04	1·16	1·40
	500	56·61** ±3·29	26·64** ±0·98	53·28 ±3·05	23·31 ±1·02	19·98 ±0·45	19·98** ±1·08	19·98 ±0·97	14·99 ±0·89	1·00	1·33
TIBA	50	59·94 ±2·08	24·98 ±0·32	53·28 ±2·62	24·98** ±1·03	26·64** ±0·72	16·65 ±0·73	26·64** ±1·23	16·65** ±1·08	1·60	1·60
	100	56·61** ±3·07	24·98 ±0·43	49·95 ±1·82	23·31 ±1·02	26·64** ±0·45	18·32 ±0·62	23·31 ±1·24	16·65** ±1·09	1·45	1·40
	200	51·62 ±2·34	22·48 ±0·75	43·29 ±1·39	26·64 ±0·98	27·64 ±0·83	18·32 ±0·63	23·31 ±1·24	14·99 ±0·65	1·50	1·50
	500	49.95 ±3·02	21·65 ±0·69	46·62 ±2·60	21·65 ±1·07	29·97 ±1·42	19·98** ±1·08	23·31 ±1·24	19·98 ±0·88	1·50	1·60

Table 2 (*Continued*)

Treatment	Concentration (ppm)	Epidermal cell size				Stomatal size				Stomatal length/breadth ratio	
		Abaxial		Adaxial		Abaxial		Adaxial			
		Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Length (μ)	Breadth (μ)	Abaxial	Adaxial
Kn	10	57·44** ±3·04	26·64** ±1·01	59·94 ±2·90	25·81** ±1·08	21·65 ±0·82	16·65 ±0·94	19·98 ±0·97	13·32 ±1·02	1·30	1·50
	25	49·95 ±2·92	24·98 ±1·05	55·78** ±3·08	24.14* ±1·09	23·31 ±1·02	16·65 ±0·94	23·31 ±0·96	13·32 ±0·43	1·40	1·75
	50	49·12 ±3·03	23·31 ±0·97	53·28 ±3·02	23·31 ±0·98	26·64** ±1·21	18·33 ±0·78	23·31 ±1·24	16·65** ±1·04	1·45	1·40
	100	43·29 ±1·07	20·32 ±0·74	51·62 ±2·62	21·65 ±0·82	27·64* ±1·09	19·98** ±0·43	26·64** ±1·42	9·99 ±0·23	1·38	2·60

* Insignificant at 1%;
** Insignificant at 1 and 5%.

4. Discussion

The formation of persistent stomatal initials, single guard cells and other abnormalities observed here agree with those reported by Gangadhara *et al* (1977) who believe that the differentiation of a meristemoid into a stomatal complex may be controlled by the specific intrinsic factors at the region of the meristemoid and these factors are disturbed by the exogenous application of growth substances which ultimately lead to such aberrant developments. The present study shows that stomatal abnormalities are induced by the exogenous application of growth substances.

From this study it is concluded that in IAA treatment stomatal frequency increased up to 200 ppm while in GA_3, TIBA and Kn it increased in all concentrations on both the leaf surfaces. The present observations regarding stomatal frequency are in agreement with those of Gangadhara and Inamdar (1975) who also reported increase in stomatal frequency by GA_3 and IAA.

From the present findings it is clear that the number of epidermal cells are more on the adaxial surface than on the abaxial surface in control. All concentrations of IAA exhibited more epidermal cells on the abaxial side than on the adaxial side. In GA_3 treatment, the number of epidermal cells present on the adaxial surface is almost double that present on the abaxial side in 50 and 500 ppm while in 100 and 200 ppm it is the same on both the surfaces. It was observed that GA_3 treatment showed a gradual decrease in the number of epidermal cells as concentration increased. Such findings have also been recorded by Murty *et al* (1976). TIBA and Kn increased the frequency of epidermal cells on both the surfaces with increase in concentration.

Solereder (1908) suggested that length to breadth ratios may give the actual shape of the stomata. According to him the range of possible variation is limited, since the shape can only be (i) broader than long, (ii) roundish, (iii) broadly elliptical, (iv) narrowly elliptical and (v) angular. In this particular plant the third and fourth categories are common for all the treatments. The second is of more restricted occurrence, while the first and fifth are not observed in any case.

Control plants have stomata of different shapes on the two surfaces, which is clear from length/breadth ratio. The shape is broadly elliptical on the abaxial side and narrowly elliptical on the adaxial side. Among all the treatments used, roundish shape of stomata are observed in GA_3 500 ppm treatment on the abaxial surface. On the abaxial surface narrowly elliptical stomata are observed in 50 and 100 ppm of IAA, 100 ppm of GA_3, 50 ppm of TIBA, while in the remaining concentrations broadly elliptical stomata are observed. On the adaxial surface narrowly elliptical stomata are found in 50 and 500 ppm of IAA, 50 ppm of TIBA and 25 ppm of kinetin, remaining concentrations showed broadly elliptical stomata. In Kn 100 ppm a special feature observed on the adaxial surface is that stomata are very narrowly elliptical, *i.e.* the length is nearly three times the breadth.

Acknowledgements

One of the authors (Vidhu) acknowledges the UGC for a fellowship.

References

Cooper M J, Digby J and Cooper P J 1972 Effects of plant hormones on the stomata of barley: a study of interaction between abscisic acid and kinetin; *Planta* **105** 43–49

Gangadhara M and Inamdar J A 1975 Action of growth regulators on the cotyledonary stomata of *Cucumis sativus* L. structure and ontogeny; *Biol. Plant.* **17** 292–303

Gangadhara M, Rao J B, Inamdar J A and Patel R M 1977 Effect of growth regulators on the structure and development of cotyledonary and hypocotyledonary stomata of *Gossypium hirsutum* L var. 'digvijay'; *Phyton* **18** 9–28

Inamdar J A 1970 Action of growth regulators on the development of stomata of *Abelmoschus esculentus* Moench; *Flora* **159** 497–502

Inamdar J A, Gangadhara M and Sanjeeva Rao M 1974 Effect of growth regulators on stomata structure and ontogeny in the cotyledons of *Cucurbita maxima* Duch; *Geobios* **1** 113–117

Kasat M L 1979 Morphogenetic effect of various growth regulators on the foliar stomata of *Vigna sinensis*; *Geophytology* **9** 102–107

Murty Y S, Prakash G and Poonia O P 1976 Effect of gibberellic acid on cotyledonary epidermis of *Crotalaria juncea* L; *Sci. Cult.* **42** 438–440

*Salisbury E J 1927 On the cause and ecological significance of stomatal frequency with special reference to woodland flora; *Philos. Trans. R. Soc. Lond.* **B216** 1–65

*Salisbury E J 1932 The interrelations of soil, climate and organisms and the use of stomatal frequency as an integrating index of the water relation of the plant; *Beih. Bot. Zool.* **99** 402–420

Sharma G K and Dunn D B 1968 Effect of environment on the cellular features in *Kalanchoe fedchenkoi*; *Bull. Torrey. Bot. Club* **95** 464–473

Solereder H 1908 *Systematic anatomy of the dicotyledons* (Oxford: Clarendon Press)

---

* Original not consulted.

Proceedings (Plant Sciences)

Volume 94, 1985

SUBJECT INDEX

Acetylene reduction
Nitrogen fixation by *Nostoc* strains 59
Achaetomium
On *Achaetomium* and a new genus Subramaniula (Ascomycota) 341
Acremonium
Fungal endophytes of forage grasses 129
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273
Additional DNA sequences
Additional DNA sequences – Topography, property, role and evolutionary significance 487
Agaricales
The relevance of chemical characters for the taxonomy of Agaricales 381
Aleuriospores
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
Alloploid
Cytology of woody species 607
Ambrosia beetles
Ambrosia beetles and their associated fungi research trends and techniques 137
Ambrosia fungi
Ambrosia beetles and their associated fungi: research trends and techniques 137
Ammonium and nitrate inhibition
Nitrogen fixation by *Nostoc* strains 59
Amphotericin B
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453
Andromedeae (Ericaceae)
The veinlet syndrome in the tribe Andromedeae (Ericaceae) 639
Anogeissus latifolia
Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.) – I. Stem-bark and root 589
Anthraquinonic pigments
The relevance of chemical characters for the taxonomy of Agaricales 381
Antifungal agents
Drechslera longirostrata (Subramanian) and other Drechslera species pathogenic to humans and animals 453
Aquatic hyphomycetes
Conidia as modified hyphae 109
Areolate
Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae 51
Arthrinium
A re-appraisal of the relationships between Arthrinium and other hyphomycetes 281
Arthro-aleuriospore
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
Ascomycetes
Meliolina subramanianii sp. nov. 347
Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* 355
Ascomycotina
Problems and prospects in the systematics of the Ascomycotina 319
Asynapsis
Cytology of woody species 607

Barren capitula
Floral differentiation and its modification 523
Basifimbria spinosa
Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete 259
Bicarbonates
Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae 45
Biochemical behaviour
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
Bioclimatic factors
Strand plant communities of the Indian subcontinent 505
Bio-ecological interactions
Management of *Rhizoctonia solani* diseases with chemicals 415
Biological species concept
The species concept in Hymenochaetaceae (Fungi: Hymenomycetes) 369
Black yeast
Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice 437
Blastoconidia
Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete 259
Blue-green algae
Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae 45

Brassica campestris
Seedling anatomy of *Brassica campestris* L 633
Breadwheat
Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L) 33
Breeding approaches
India's changing agriculture: Its management, genetic vulnerability and diversification 465
Bulbostylis
Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memorian optimi Professoris Swamy 11

Cajanus cajan
Foliar application of potassium on the growth and yield components of Pigeonpea (*Cajanus cajan* (L.) Millsp.) 671
Calendula
Floral differentiation and its modification 525
Callus cultures
Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L) 33
Capsicum
Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations 663
Carbonates
Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae 45
Caryophyllales
Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae 51
Cereal root rots
Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia 91
Chaetomium
On *Achaetomium* and a new genus Subramaniula (Ascomycota) 341
Chelisporium
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 229
Chemotaxis
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475
Chemotaxonomy
The relevance of chemical characters for the taxonomy of Agaricales 381
Chenopodium
Floral differentiation and its modification 525
Chieroconium
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 229
Chlorflurenol
Floral differentiation and its modification 525
Chromosome number
Cytology of woody species 607
Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) 619
Chromosome structure
Additional DNA sequences – Topography, property, role and evolutionary significance 487
Chrysanthemum
Floral differentiation and its modification 525
C. lichenicola
Cylindrocarpon species associated with mycotic keratitis 433
Classification
A re-appraisal of the relationships between Arthrinium and other hyphomycetes 281
Problems and prospects in the systematics of the Ascomycotina 319
Combretaceae
Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.) – I. Stem-bark and root 589
Compositae
Development of male and female gametophytes and integument initiation in *Prenanthes brunoniana* Wall. (Compositae) 553
Condensed chromatin
Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation 539
Conidial ontogenesis
Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice 437
Conidiogenesis
Conidia as modified hyphae 209
A re-appraisal of the relationships between Arthrinium and other hyphomycetes 281
Conidiophores
Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete 259
Coprophilous Hyphomycete
Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete 259
Cordyceps cantharelloides
New and rare entomogenous fungi from Amazonia 309
Cortinarius
The relevance of chemical characters for the taxonomy of Agaricales 381
C. rhizophorae
Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* 355
Cyanobacteria
Nitrogen fixation by *Nostoc* strains 59

Cytokinesis
Cytology of woody species 607
Cytomixis
Cytology of woody species 607

Dactuliophora elongata
Identity of dactuliophora leaf spot of pearl millet 407
Dactuliophora leaf spot
Identity of dactuliophora leaf spot of pearl millet 407
Dematiaceae
Porosubramaniana, a new genus of lignicolous Hyphomycetes 253
Dematiaceous Hyphomycetes
Pigment localization in Dematiaceous Hyphomycetes and the segregation of *Pseudogliomastix* gen. nov. from *Acremonium* 273
Dermatophytes
Ecology of keratinophilic fungi 149
Dermocybe
The relevance of chemical characters for the taxonomy of Agaricales 381
Desynapsis
Cytology of woody species 607
Dhava
Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.) – I. Stem-bark and root 589
Dictyosporium
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 229
Digitodesmium
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 229
Dinitroaniline
Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species 41
Directional movement
Monitoring of wheat rusts in the Indian subcontinent 387
Dissemination
Monitoring of wheat rusts in the Indian subcontinent 387
DNA sequences
Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation 539
Dravanti
Pharmacognostic studies on 'Dravanti' Part-I *Jatropha curcas* Linn. 65
Drechslera species
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453
Edaphic factors
Strand plant communities of the Indian su continent 5
Eleocharis
Studies in Cyperaceae: XIX. Species novaru Cyperacerum in memoriam optimi Professo Swamy
Endophytes
Fungal endophytes of forage grasses 1
Entomogenous fungi
New and rare entomogenous fungi fro Amazonia 3
Epidemiology
Monitoring of wheat rusts in the Indian su continent 3
Extra-cellular polysaccharides
Immunogenic analysis of extra-cellular polysa charide of rhizobia collected from South Indi soils 5
Eugymnohymenial ascomata
Pseudascozonus, a new genus of Pezizales 3
Euphorbiaceae
Pharmacognostic studies on 'Dravanti' Par *Jatropha curcas* Linn.

Farming systems
India's changing agriculture: Its managemer genetic vulnerability and diversification 4
Festuca
Fungal endophytes of forage grasses 1
Fimbristylis
Studies in Cyperaceae: XIX. Species novaru Cyperacearum in memoriam optimi Professo Swamy
Fimicolous fungi
Pseudascozonus, a new genus of Pezizales 3
Flagellates
Photodynamically induced behavioural respo ses of the colourless flagellate *Polytomella magn* Effects methylene blue 4
Floral differentiation
Floral differentiation and its modification 5
Floral meristem
Floral differentiation and its modification 5
Floral morphogenesis
Floral differentiation and its modification 5
Floral organogenesis
Floral differentiation and its modification 5
Flower drop
Foliar application of potassium on the gro and yield components of pigeonpea (*Caja cajan* (L.) Millsp.)
Foliar application
Foliar application of potassium on the gro and yield components of pigeonpea (*Caja cajan* (L.) Millsp.)

Foliar sclereids
Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight 655
Fruit bodies
Factors affecting the occurrence of fruitbodies of fungi forming sheathing(ecto-) mycorrhizas with roots of trees 111
Fuirena
Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memoriam optimi Professoris Swamy 11
Fungal penetration
Specificity and penetration of tissues by mycorrhizal fungi 99
Fungal spores
Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) 165
Fungal taxonomy
Two new hyphomycetes from Ethiopia 269
On *Achaetomium* and a new genus Subramaniula (Ascomycota) 341
Fungi
Factors affecting the occurrence of fruitbodies of fungi forming sheathing(ecto-) mycorrhizas with roots of trees 111
Fungal endophytes of forage grasses 129
Problems and prospects in the systematics of the Ascomycotina 319
Meliolina subramanianii sp. nov. 347
Fungicides
Management of *Rhizoctonia solani* diseases with chemicals 415
Fusarium solani
Cylindrocarpon species associated with mycotic keratitis 433
Fuseaux
Physiological studies of the Gymnoascaceae with an overview on their ecology 197

Gaeumannomyces graminis var. *tritici*
Effect of soil texture on microbial abbreviation of saprophytic survival by the take-all fungus of wheat 85
Gametophytes
Development of male and female gametophytes and integument initiation in *Prenanthes brunoniana* Wall. (Compositae) 553
GC content
Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice 437
Genetic elements
Additional DNA sequences – Topography, property, role and evolutionary significance 487
Genetic potentials
India's changing agriculture: Its management, genetic vulnerability and diversification 465
Germination
Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) 165
Gibberellic acid
Floral differentiation and its modification 525
Gliomastix
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273
Glycogen
Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae 45
Growth
Seasonal growth, reproduction and spore shedding in *Pterocladia heteroplatos* 627
Growth substances
Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations 663
Effect of different growth substances on the foliar stomata of *Tagetes erecta* L 683
Gymnothecium
Physiological studies of the Gymnoascaceae with an overview on their ecology 197

Halophytes
Strand plant communities of the Indian subcontinent 505
Helicorhoidion
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 229
Helicosporous hyphomycetes
On the anamorph genus *Cirrenalia* 245
Hirsutella subramanianii
New and rare entomogenous fungi from Amazonia 309
Hydrobiological studies
Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) 583
Hymenochaetaceae
The species concept in Hymenochaetaceae (Fungi, Hymenomycetes) 369
Hyphal morphogenesis
Conidia as modified hyphae 209
Hyphomycetes
Two new hyphomycetes from Ethiopia 269
A re-appraisal of the relationships between Arthrinium and other hyphomycetes 281

Immunogenicity
Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils 575

Indian agriculture
India's changing agriculture: Its management, genetic vulnerability and diversification 465
Inflorescence
Floral differentiation and its modification 525
Infrared analysis
Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils 575
Inoculum
Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia 91
Integument initiation
Development of male and female gametophytes and integument initiation in *Prenanthes brunoniana* Wall. (Compositae) 553
Interphase nuclear structure
Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation 539
Itraconazole
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453

Jatropha curcas
Pharmacognostic studies on 'Dravanti' Part-I *Jatropha curcas* Linn. 65

Keratin
Ecology of keratinophilic fungi 149
Keratinophilic fungi
Ecology of keratinophilic fungi 149
Ketoconazole
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453

Labiatae
Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) 619
Laccase
Survey of some Indian soils for laccase producing fungi and their lignin degrading ability 567
Lakes
Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) 583
Lichena
Problems and prospects in the systematics of the Ascomycotina 319
Lignicolous Hyphomycetes
Porosubramaniana, a new genus of lignicolous Hyphomycetes 253
Lignin
Survey of some Indian soils for laccase producing fungi and their lignin degrading ability 567
Lipase
Enzymes from thermophilic fungi: Proteases and lipases 175
Lolium
Fungal endophytes of forage grasses 129
Long-day plants
Floral differentiation and its modification 525

Management
Monitoring of wheat rusts in the Indian subcontinent 387
Mangrove
Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* 355
Marine fungi
On the anamorph genus *Cirrenalia* 245
Meliolina
Meliolina subramanianii sp. nov. 347
Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* 355
Methylene blue
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475
Microbial hydrolases
Enzymes from thermophilic fungi: Proteases and lipases 175
Microbial rennet
Enzymes from thermophilic fungi: Proteases and lipases 175
Micromorphology
Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae 51
Microspecies
The species concept in Hymenochaetaceae (Fungi, Hymenomycetes) 369
Molluginaceae
Seedcoat micromorphology of Caryophyllales: Observations of some Molluginaceae 51
Monilioid conidia
Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) 165
Monosaccharide composition
Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils 575
Mutualism
Ambrosia beetles and their associated fungi: research trends and techniques 137
Mycorrhiza
Specificity and penetration of tissues by mycorrhizal fungi 99

Mycorrhizas
Factors affecting the occurrence of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with roots of trees 111
Mycoses
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453
Mycotic keratitis
Cylindrocarpon species associated with mycotic keratitis 433

Neurotropism
Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice 437
Nitrite
Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species 41

Opportunistic
Physiological studies of the Gymnoascaceae with an overview on their ecology 197

Parameter
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
Pasture
Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia 91
Pearl millet
Identity of dactuliophora leaf spot of pearl millet 407
Pericarp
The pericarpic structure of *Withania somnifera* (L.) Dunal 677
Petiolar explant abscission
Abscission responses of petiolar explants of *Capsicum* varieties to treatments with growth substances and their combinations 663
Petrophile
Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight 655

Pezizales
Pseudascozonus, a new genus of Pezizales 363
Pharmacognosy
Pharmacognostic studies on 'Dravanti' Part-I *Jatropha curcas* Linn. 65
Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.)–I. Stem-bark and root 589
Photodispersal
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475
Photodynamic action
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475
Phytoplankton biomass
Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) 583
Pigmentation
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273

Placentae
The pericarpic structure of *Withania somnifera* (L.) Dunal 677
Plant height
Foliar application of potassium on the growth and yield components of pigeonpea (*Cajanus cajan* (L.) Millsp.) 671
Plant regeneration
Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L) 33
Pod structure
The structure of the pod in two species of *Prosopis* 1

Polarity
Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) 165
Polybasic
Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) 619
Polymorphism
Significance of seed coat pattern in *Vigna radiata* var. *sublobata* 561
Polyploidy
Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) 619
Polytomella magna
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475
Ponds
Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) 583
Porosubramaniana gen. nov.
Porosubramaniana, a new genus of lignicolous Hyphomycetes 253
Porosubramaniana moniliformis sp. nov.
Porosubramaniana, a new genus of lignicolous Hyphomycetes 253

cation
nitoring of wheat rusts in the Indian sub-
tinent 387
nthes brunoniana
velopment of male and female gametophytes
integument initiation in *Prenanthes bruno-*
na Wall. (Compositae) 553
pis
structure of the pod in two species of *Prosopis*
1
aceae
ology and taxonomic value of foliar sclereids
he proteaceae III. *Petrophile* R. Br. ex. Knight
655
ases
zymes from thermophilic fungi: Proteases and
ses 175
mophytes
and plant communities of the Indian sub-
tinent 505
ascozonus
udascozonus, a new genus of Pezizales 363
ogliomastix
ment localization in Dematiaceous
phomycetes and the segregation of
udogliomastix gen. nov. from Acremonium
273
cladia heteroplatos
sonal growth, reproduction and spore shed-
g in *Pterocladia heteroplatos* 627

uetmycelia
siological studies of the Gymnoascaceae with
overview on their ecology 197
duction
sonal growth, reproduction and spore shed-
g in *Pterocladia heteroplatos* 627
sal to vegetative growth
ral differentiation and its modification 525
bial isolates
nunogenic analysis of extra-cellular polysac-
ride of rhizobia collected from South Indian
s 575
ctonia solani
nagement of *Rhizoctonia solani* diseases with
micals 415
phora
yosporella rhizophorae gen. et sp. nov.
assariaceae), a marine Ascomycete from
zophora mangle 355

phytic survival
ct of soil texture on microbial abbreviation of
rophytic survival by the take-all fungus of
at 85
id idioblasts
veinlet syndrome in the tribe Andromedeae
(Ericaceae) 639
Scleria
Studies in Cyperaceae: XIX. Species novarum
Cyperacearum in memoriam optimi Professoris
Swamy 11
Seasonal changes
Seasonal growth, reproduction and spore shed-
ding in *Pterocladia heteroplatos* 627
Seedcoat
Seedcoat micromorphology of Caryophyllales:
Observations on some Molluginaceae 51
Seed coat pattern
Significance of seed coat pattern in *Vigna radiata*
var. *sublobata* 561
Seedling anatomy
Seedling anatomy of *Brassica campestris* L 633
Seedling rot
Management of *Rhizoctonia solani* diseases with
chemicals 415
Selection species concept
The species concept in Hymenochaetaceae
(Fungi, Hymenomycetes) 369
Selective herbicide
Accumulation of nitrite as influenced by fluchlo-
ralin and benthiocarb in two tropical weed species
41
Short-day plants
Floral differentiation and its modification 525
Sibling species
The species concept in Hymenochaetaceae
(Fungi, Hymenomycetes) 369
Sinapis
Floral differentiation and its modification 525
Sirothecium
Notes on some deuteromycete genera with
cheiroid or digitate brown conidia 229
Soils
Survey of some Indian soils for laccase producing
fungi and their lignin degrading ability 567
Spacer
Additional DNA sequences – Topography, pro-
perty, role and evolutionary significance 487
Specificity
Specificity and penetration of tissues by mycor-
rhizal fungi 99
Spinacea
Floral differentiation and its modification 525
Spore shedding
Seasonal growth, reproduction and spore shed-
ding in *Pterocladia heteroplatos* 627
Sporulation
Effect of carbonate and bicarbonate on growth
and sporulation in two blue-green algae 45
Stem-bark and root
Pharmacognostic studies on "Dhava"
(*Anogeissus latifolia* Bedd.) – I. Stem-bark and
root 589

tomata
Effect of different growth substances on the foliar stomata of *Tagetes erecta* L. 683
trand plant communities
Strand plant communities of the Indian subcontinent 505
tyryl-pyrones
The relevance of chemical characters for the taxonomy of Agaricales 381
ubramaniula
On *Achaetomium* and a new genus Subramaniula (Ascomycota) 341
ymbiosis
Specificity and penetration of tissues by mycorrhizal fungi 99
Ambrosia beetles and their associated fungi: research trends and techniques 137
ystematics
Problems and prospects in the systematics of the Ascomycotina 319
ystematic position
Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae 51
yzygium
Meliolina subramanianii sp. nov. 347

agetes
Floral differentiation and its modification 525
agetes erecta
Effect of different growth substances on the foliar stomata of *Tagetes erecta* L 683
ake-all fungus
Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia 91
axonomic and diagnostic value
The veinlet syndrome in the tribe Andromedeae (Ericaceae) 639
axonomic significance
The structure of the pod in two species of *Prosopis* 1
axonomy
Conidia as modified hyphae 209
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273
eleomorph
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
errestrial fungi
On the anamorph genus *Cirrenalia* 245
helebolaceae
Pseudascozonus, a new genus of Pezizales 363
hermophilic fungi
Enzymes from thermophilic fungi: Proteases and lipases 175
Thiocarbamate
Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species 41
Transposons
Additional DNA sequences – Topography, property, role and evolutionary significance 487
Triticum aestivum
Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L) 33
Tuberculate
Seedcoat micromorphology of Caryophyllales: Observations on some Molluginaceae 51
Type studies in fungi
The species concept in Hymenochaetaceae (Fungi, Hymenomycetes) 369
Typology
Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight 655

Ultrastructure
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273
Floral differentiation and its modification 525

Vaginae
Cylindrocarpon species associated with mycotic keratitis 433
Vegetative anatomy
Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memoriam optimi Professoris Swamy 11
Vein endings
The veinlet syndrome in the tribe Andromedeae (Ericaceae) 639
Vigna radiata var. *sublobata*
Significance of seed coat pattern in *Vigna radiata* var. *sublobata* 561
Virescent inflorescence
Floral differentiation and its modification 525

Wallrothiella
Pigment localization in Dematiaceous Hyphomycetes and the segregation of Pseudogliomastix gen. nov. from Acremonium 273
Wheat rusts
Monitoring of wheat rusts in the Indian subcontinent 387
Withania somnifera
The pericarpic structure of *Withania somnifera* (L.) Dunal 677

Wood
Survey of some Indian soils for laccase producing fungi and their lignin degrading ability 567

Xanthium
Floral differentiation and its modification 525

Yield components
Foliar application of potassium on the growth and yield components of pigeonpea (*Cajanus cajan* (L.) Millsp.) 671

AUTHOR INDEX

Arora D S
Survey of some Indian soils for laccase producing fungi and their lignin degrading ability 567
Babber S
Seedling anatomy of *Brassica campestris* L 633
Babu C R
see Ignacimuthu S 561
Bai R Kasturi
Immunogenic analysis of extra-cellular polysaccharide of rhizobia collected from South Indian soils 575
Batra L R
Ambrosia beetles and their associated fungi: research trends and techniques 137
Bhat D J
Two new hyphomycetes from *Ethiopia* 269
Bievre Claude de
see Drouhet Edouard 453
Bir S S
see Singhal V K 607
Cytological studies on members of family Labiatae from Kodaikanal and adjoining areas (South India) 619
Boekhout T
see Gams W 273
Booth C
Cylindrocarpon species associated with mycotic keratitis 433
Buffin Nathalie
Basifimbria spinosa, a new pleoanamorphic coprophilous hyphomycete 259

Chouhan Satish
see Johri Bhavdish N 175
Clayton Y M
see Booth C 433
Cole Garry T
Fungal endophytes of forage grasses 129

Dave Y S
The pericarpic structure of *Withania somnifera* (L.) Dunal 677
Descals E
Conidia as modified hyphae 209
Drouhet Edouard
Drechslera longirostrata (Subramanian) and other *Drechslera* species pathogenic to humans and animals 453

Evans Harry C
see Samson Robert A 309

Fleming L V
see Last F T 111

Gams W
Pigment localization in Dematiaceous hyphomycetes and the segregation of Pseudogliomastix gen. nov. from *Acremonium* 273
Garg A P
Ecology of keratinophilic fungi 149
Garg Raj
see Sarma T A 45
Garrett S D
Effect of soil texture on microbial abbreviation of saprophytic survival by the take-all fungus of wheat 85
Gill B S
see Singhal V K 607
Goos R D
On the anamorph genus *Cirrenalia* 245
Gopinathan M C
see Sivarajan V V 51
Gouri Rani Ghosh
Physiological studies of the Gymnoascaceae with an overview on their ecology 197
Govindarajalu E
Studies in Cyperaceae: XIX. Species novarum Cyperacearum in memoriam optimi Professoris Swamy 11
Grover Rajendra K
Management of *Rhizoctonia solani* diseases with chemicals 415
Gupta R C
Pharmacognostic studies on 'Dravanti' part-I *Jatropha curcas* Linn. 65
Pharmacognostic studies on "Dhava" (*Anogeissus latifolia* Bedd.) – I. Stem-bark and root 589

Harley J L
Specificity and penetration of tissues by micorrhizal fungi 99
Hawksworth D L
Problems and prospects in the systematics of the Ascomycotina 319
Hegde G R
Comparison of phytoplankton biomass in four water bodies of Dharwad, Karnataka State (India) 583
Hennebert G L
see Buffin Nathelie 259
Hughes S J
Meliolina subramanianii sp. nov. 347

Ignacimuthu S
Significance of seed coat pattern in *Vigna radiata* var. *sublobata* 561

Jain H K
India's changing agriculture: Its management, genetic vulnerability and diversification 465
Jain Sanjay
see Johri Bhavdish N 175
Jan Kohlmeyer
Caryosporella rhizophorae gen. et sp. nov. (Massariaceae), a marine Ascomycete from *Rhizophora mangle* 355
Jayasankari N
Nitrogen fixation by *Nostoc* strains 59
Johri Bhavdish N
Enzymes from thermophilic fungi: Proteases and lipases 175
Joshi C P
see Shubhada Patankar 539
Joshi L M
Monitoring of wheat rusts in the Indian subcontinent 387

Kaliaperumal N
Seasonal growth, reproduction and spore shedding in *Pterocladia heteroplatos* 627
Kataria Hans R
see Grover Rajendra K 415
Kothari I L
see Rangayya S 1

Last F T
Factors affecting the occurrence of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with roots of trees 111

Maria Häder
see Nultsch Wilhelm 475
Meher-Homji V M
see Rao T Ananda 505
Menon A R S
see Dave Y S 677
Minter David William
A re-appraisal of the relationships between *Arthrinium* and other hyphomycetes 281
Miyaji M
Conidial ontogenesis of pathogenic black yeasts and their pathogenicity for mice 437
Mohan Ram H Y
see Usha Rao I 525
Moser Meinhard
The relevance of chemical characters for the taxonomy of agaricales 381
Mrinal Bhave
see Shubhada Patankar 539
Mukerji K G
see Garg A P 149
Murty Y S
see Singh Karan 663
see Vidhu 683

Nishimura K
see Miyaji M 437
Nultsch Wilhelm
Photodynamically induced behavioural responses of the colourless flagellate *Polytomella magna*: Effects of methylene blue 475

Parmasto E
The species concept in Hymenochaetaceae (Fungi, Hymenomycetes) 369
Patel N D
see Dave Y S 677
Pillai A
see Babber S 633
Pirozynski K A
see Hughes S J 347
Prasad P
see Ravindranath N N V S 671
Pugh G J F
see Garg A P 149

Raju P N
see Bai R Kasturi 575
Ranade S A
see Shubhada Patankar 539
Rangayya S
The structure of the pod in two species of *Prosopis* 1
Ranjekar P K
see Shubhada Patankar 539
Rao K R
see Reddy N V R 41
Rao P S
see Susan Eapen 33

Rao T Ananda
Strand plant communities of the Indian subcontinent 505
The veinlet syndrome in the tribe Andromedeae (Ericaceae) 639
Typology and taxonomic value of foliar sclereids in the Proteaceae III. *Petrophile* R. Br. ex Knight 655
Rao K V Madhava
see Ravindranath N N V S 671
Rao M Umamaheswara
see Kaliaperumal N 627
Ravindranath N N V S
Foliar application of potassium on the growth and yield components of pigeonpea (*Cajanus cajan* (L.) Millsp.) 671
Reddy N V R
Accumulation of nitrite as influenced by fluchloralin and benthiocarb in two tropical weed species 41
Rowland I
see Sivasithamparam K 91

Saggoo M I S
see Bir S S 619
Samson Robert A
New and rare entomogenous fungi from Amazonia 309
Sandhu D K
see Arora D S 567
Sarma T A
Effect of carbonate and bicarbonate on growth and sporulation in two blue-green algae 45
Satyanarayana N V
see Ravindranath N N V S 671
Seema Wahab
see Drouhet Edouard 453
Shah G L
see Rangayya S 1
Shanmugasundaram S
see Jayasankari N 59
Sharma Arun Kumar
Additional DNA sequences – Topography, property, role and evolutionary significance 487
Sharma R K
see Vidhu 683
Shubhada Patankar
Interphase nuclear structure in plants: role of nuclear DNA content and highly repeated DNA sequences in chromatin condensation 539
Silpi Das
see Rao T Ananda 655
Singh D V
see Joshi L M 387
Singhal V K
Cytology of woody species 607
Singh Karan
Abscission responses of petiolar explants of *capsicum* varieties to treatments with growth substances and their combinations 66
Sivarajan V V
Seedcoat micromorphology of Caryophyllales
Observations on some Molluginaceae 5
Sivasithamparam K
Propagule behaviour of the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) at a field site in Western Australia 9
Sood Sarvesh Kumar
Development of male and female gametophyte and integument initiation in *Prenanthes brunoniana* Wall. (Compositae) 55
Srivastava K D
see Joshi L M 38
Sudha Gandotra
see Garg A P 14
Susan Eapen
Factors controlling callus initiation, growth and plant regeneration in breadwheat (*Triticum aestivum* L.) 3
Sutton B C
Notes on some deuteromycete genera with cheiroid or digitate brown conidia 22
Swapna Chakraborti
see Rao T Ananda 63
see Rao T Ananda 65

Thakur Tejinder Singh
see Sood Sarvesh Kumar 55
Turian Gilbert
Polarizing processes for germ tube outgrowth from monilioid fungal spores (*Neurospora* conidial model) 16
Tyagi P D
Identity of dactuliophora leaf spot of pearl millet 40

Usha Rao I
Floral differentiation and its modification 52
Usherwood M
see Booth C 43

van Brummelen J
Pseudascozonus, a new genus of Pezizales 36
Vera Holubova-Jechova
Porosubramaniana, a new genus of lignicolous hyphomycetes 25
Vidhu
Effect of different growth substances on the foliar stomata of *Tagetes erecta* L 68
von Arx J A
On *Achaetomium* and a new genus *Subramaniula* (Ascomycota) 3

White James F
see Cole Garry T 1